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LONGEVITY OF CONGELATIONS OF BULB NEMATODE, *DITYLENCHUS DIPSACI* (KUHN) FILIPJEV, FROM NARCISSUS[1]

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Little experimental evidence has been published concerning the dormancy period of the bulb nematode of narcissus, but Corder (1) in 1933, and McBeth (2) in 1937 working with the same samples at Salt Lake City, have published data on the longevity of the bulb nematode in various other plant material. They presented evidence showing that the survival of the bulb nematode in dried plant tissue varies with the host and with the tissue; thus in alfalfa buds the nematodes survive less than 3 years; in alfalfa seed, 5 years; in long leaf plantain, 6 years; in garlic, 7 years; and in oats and teasel over 9 years.

The bulb nematodes that infest narcissus are frequently first found in the leaves. From thence they migrate downwards to the bulb. Reproduction seems to be most rapid in the scales, and finally they reach the basal plate between August and October. Here the nematodes do not appear to mature for the populations consist chiefly of preadults. Apparently the nematodes leave the bulb in the preadult stage if left in the ground. In stored bulbs the nematodes emerge from fissures between the basal plate and bulb scales but, unable to go further, they gather into masses, each worm eventually becoming coiled and immobile, and in time the aggregations assume a curd-like consistency. These congelations from the narcissus bulb are the most convenient and abundant sources of bulb nematode material for experimental work and they were used in the following study of viability in relation to time.

EXPERIMENTAL

Congelations of the bulb nematode were collected from narcissus bulbs during September to October of 1937, 1938, 1939 and 1940, and were stored in glass vials. At the time of the present tests they have dormancy periods of $1\frac{1}{4}$ to $4\frac{1}{4}$ years. The tests were made by placing small whole congelations of 1000 to 2000 worms in 5 cc. of tap water in Syracuse dishes. The room temperature was approximately 70° F. The suspensions were examined for motility under a binocular microscope at regular intervals up to 72 hours. Since there might be considerable variations in mortality of different congelations of the same year, owing to the unprotected state of

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the nematodes and varying environments at the time of collection, six series of tests were conducted, each replication consisting of four plates and one small congelation of each collection. The data are presented in Table 1.

TABLE 1.—THE EFFECT OF LENGTH OF DORMANCY ON THE SURVIVAL OF BULB NEMATODE CONGELATIONS FROM NARCISSUS BULBS

Year collected	No. of years dormant	Test series No.	Percentage of nematodes motile in relation to time in water				Survival average of 6 tests	Mortality
			3 hr.	6 hr.	24 hr.	72 hr.		
			%	%	%	%	%	%
1940	1¼	1	18	40	70	72		
		2	10	20	55	55		
		3	20	85	85	82		
		4	16	88	88	95		
		5	10	60	84	84		
		6	14	58	88	90	79.6	20.4
1939	2¼	1	1	23	25	22		
		2	0	9	15	17		
		3	0	12	13	15		
		4	0	14	14	14		
		5	0	0.1	0.1	0.1		
		6	0	4	18	18	14.3	85.7
1938	3¼	1	0	7	10	10		
		2	0	3	5	8		
		3	0	0	0.1	0.3		
		4	0	0	0	0		
		5	0	3	3	5		
		6	0	2	3	4	4.5	95.5
1937	4¼	1	0	0	0	0		
		2	0	0	0	0		
		3	0	0	0	0		
		4	0	0	0	0		
		5	0	0	0	0		
		6	0	0	0	0	0	100.0

The data reveal a comparatively low mortality, 5 to 20% in the first year, a considerable increase in the second year to 85.7%, and in the third year to 95.5%. No living nematodes were seen among over 10,000 examined, after four years dormancy. For experimental purposes, even congelations of two years are hardly suitable in view of the few living nematodes left.

The revival rate was generally in direct relation to the length of dormancy. One-year-old congelations commence to show signs of motility 2 to 3 hours after they are placed in water, two-year-old congelations 3 to 4 hours, and the maximum revival in both cases requires about 24 hours. Three-year-old congelations become motile in about six hours and maximum revival requires 48 to 72 hours. In comparison with the bulb nematodes in dried plant tissue, these unprotected congelations from narcissus appear

to survive a relatively short time, only about equal to those in alfalfa bud. The nematodes in alfalfa bud survived less than three years, this value being the lowest of the several populations reported by McBeth (2).

SUMMARY

The bulb nematodes that develop in narcissus bulbs and eventually emerge through fissures between the basal plate and bulb scales, form congelations. Experiments show that the mortality of the nematodes in these congelations is influenced by the length of dormancy; thus after one year the mortality averages 20%, two years 85.7%, three years 95.5%, and four years 100%.

The revival rate was in direct relation to length of dormancy. One-year congelations commence to show motility in two to three hours; two-year congelations in three to four hours; both attain maximum revival in 24 hours. Three-year congelations show motility in about 6 hours and maximum revival requires 48 to 72 hours. These congelations of bulb nematode from narcissus survive a relatively short time (three years) compared with the bulb nematode in dried plant tissue of other crops (two to over nine years).

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THE RELATION OF CLAY AND ORGANIC MATTER TO SOIL MOISTURE EQUILIBRIUM POINTS[1]

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Wide variations in the mechanical composition and organic matter content of soils are generally encountered within any large area. The texture of the soil may vary from sand or gravel to heavy clay. If the soils in the area belong to several of the major world groups, their organic matter content may differ as much as 8 to 10%. In Saskatchewan there is a progressive increase in organic matter content from the brown soils in the southwest of the province to the black soils farther north and east, followed by a decrease in the grey wooded (podsolic) soils of the forested region (Figure 1). The fact that soils with higher clays contents are generally more resistant to drought is well known, and among the many beneficial effects attributed to the presence of a sufficient supply of organic matter in the soil, the improvement in moisture retaining capacity is commonly given an important position.

The present study was carried on for the purpose of obtaining information on the following points: (1) A general comparison of moisture retention by different types of soils; (2) the relation of clay and organic matter to soil moisture equilibrium points, with special attention given to Hygroscopic Coefficient, Permanent Wilting Point and Moisture Equivalent; (3) the indirect calculation of the permanent wilting point, either by relationship to the physical composition of the soil, or from its relationship to the hygroscopic coefficient.

REVIEW OF LITERATURE

Numerous investigations dealing with the relation of soil composition to soil moisture have directed attention to the importance of the relative percentages of sand, silt and clay. Somewhat less attention has been given to the role of organic matter in its effect on the state of moisture in the soil. Robinson (17) suggests that "More work is desirable to establish the correlation, which undoubtedly exists, between hygroscopicity and organic matter content. The data hitherto obtained do not permit the accurate expression of hygroscopic capacity as a function of clay and organic matter, but merely indicate correlations." Keen (10) states, "Colloidal material exists in the soil, derived both from the humus and clay fraction, and exerts in most cases a controlling influence on the water relationships." To substantiate this Keen ignited a soil at dull red heat, and found that its evaporation curve was almost the same as for pure

[1] Contribution from the Department of Soils, University of Saskatchewan, in partial fulfilment of the requirements for the degree of Master of Science.

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sand. Alway and Neller (1) found that added organic matter caused an increase in free moisture on field plots. Muller (13) observed similar results. Soils receiving an annual application of organic matter showed an increase in moisture retention over untreated plots. Keen and Coutts (11) studied the moisture contents of different soils in equilibrium with an atmosphere of 50% relative humidity. At this moisture content the clay was mainly responsible for moisture retention. On the other hand Coutts (7) in a later work obtained a much higher correlation between moisture content at 70% relative humidity and organic matter. Middleton (12) states, "The presence of considerable amounts of organic matter tends to increase the moisture equivalent and disturb the relation between it and mechanical analysis." Smith (18) found that several soils having the same mechanical analysis gave very different results for moisture equivalent. This variation in results may have been due in part to variations of organic matter content.

Considering the quotations given above, it appears that soil organic matter must exert an influence on moisture relationships within the hygroscopic moisture range and within the range of available moisture, and these relationships cannot be overlooked when studying soil moisture equilibrium points.

Hygroscopic coefficient has been used as a measure of the colloidal material of the soil. It has been defined by Puri (15) as "the amount of moisture that a soil will hold in equilibrium with an atmosphere of 50% relative humidity." This equilibrium point occurs on the steepest part of the vapour pressure-moisture curves as determined by Puri, Crowther and Keen (14). At this point there will be very little variation in moisture adsorbed with changes in the relative humidity of the atmosphere.

Keen and Coutts (11) obtained a good correlation between clay and moisture content at 80% relative humidity. Statistical analysis of their data indicated that the amount of organic matter present might have had an influence.

Puri (16) established an empirical equation whereby he estimated clay ($<$ .001 mm.) content from percentage moisture adsorbed by a soil in equilibrium with an atmosphere of 70% relative humidity. His errors of estimate varied from $-$ 7.4% to $+$ 8.6%.

It is apparent from these studies that there is a close relationship between hygroscopic moisture and soil colloidal material. As yet no standard definition of hygroscopic coefficient has been accepted.

The wilting coefficient or permanent wilting point was first proposed by Briggs and Shantz (6). They showed that the wilting point expressed as a percentage based on oven dry soil is practically the same for all plants on the same soil.

Veihmeyer and Hendrickson (21) state that the wilting coefficient is independent of environmental conditions and is constant for all plants on the same soil type.

Baver (4) considers that all indirect methods of determining the wilting coefficient are unreliable. However, many workers continue to use indirect methods as a means of obtaining some indication of the soil moisture at this important point. Direct experimental determinations of the wilting coefficient require considerable time and careful supervision, in order that reliable results may be obtained.

Many attempts have been made to formulate empirical equations relating the moisture equivalent to mechanical composition of the soil, in order to use it as an index of texture. Equations of this type were formulated by Briggs and McLane (5), Briggs and Shantz (6), Alway and Russel (3) and Middleton (12). Alway and Russel (3) suggest that "if mechanical analysis is to be used as an indirect means of calculating the moisture equivalent, it will at least be necessary to employ a different formula for widely differing soil types."

The general conclusions seem to be that there is no close relationship between texture and moisture equivalent. However, since the introduction of the pF scale in soil moisture relationships, further interest in the determining of the moisture equivalent is appearing.

MATERIALS AND METHODS

Analytical Methods

Mechanical analysis was carried out according to Troell's (19) modification of the Robinson pipette method. The soil separates were taken off on the basis of the "International System."

Organic matter was calculated using the factor 1.72 × organic carbon. A modification of the Winters and Smith (23) method was used to determine organic carbon (dry combustion). Since no oxygen tank was readily available the combustion was carried on in the presence of CO_2 free air, using manganese dioxide to hasten oxidation. The carbon dioxide evolved was absorbed in a standard solution of potassium hydroxide.

A modification of the vapour pressure technique of Puri, Crowther and Keen (14) was used for the determination of the hygroscopic coefficient. A vacuum desiccator was used but a saturated solution of potassium nitrite, which gives an atmosphere of 45% relative humidity at 20° C., was substituted for the sulphuric acid-water mixture because it eliminated the necessity of frequently changing the solution. All samples were first dried over concentrated sulphuric acid (in a vacuum desiccator) before carrying out the determination of the hygroscopic coefficient.

A slight modification of the Work and Lewis (24) method was used for the determination of the wilting coefficient. Dwarf sunflowers were the plants used in this determination and proved very satisfactory as indicators of both temporary and permanent wilting.

The method of Briggs and McLane (5) was used in the determination of the mosture equivalent. The saturated soil was centrifuged in perforated cups at a speed of 2440 revolutions, which applies a force of 1000 times that of gravity to the soil water.

TABLE 1.—DESCRIPTION OF SAMPLES

Lab. No.	Soil Zone	Texture	Series
39– 5	Black (deep)	Fine Sandy Loam	Meota
39– 6	Black (deep)	Fine Sandy Loam	Meota
39– 7	Intrazonal (Glei Podzol)	Loam	——
39– 8	Black (shallow)	Clay	Indian Head
39– 9	Black (shallow)	Clay	Indian Head
39–10	Black (shallow)	Clay Loam	Oxbow
39–11	Black (shallow)	Loam	Oxbow
39–12	Dark Brown	Clay Loam	Weyburn
39–13	Dark Brown	Loam	Weyburn
39–14	Dark Brown	Light Loam	Weyburn
39–15	Dark Brown	Loam	Weyburn
39–16	Dark Brown	Light Loam	Weyburn
39–20	Dark Brown	Loam	Weyburn
39–21	Dark Brown	Heavy Clay	Regina
39–22	Dark Brown	Clay Loam	Weyburn
39–27	Dark Brown	Loam	Cypress
39–28	Brown	Light Loam	Haverhill
39–29	Brown	Loam	Fox Valley
39–31	Brown	Very Fine Sandy Loam	Hatton
39–32	Brown	Heavy Clay	Sceptre
39–33	Brown	Loam	Fox Valley
H– 1	Black (deep)	Light Loam	Yorkton
H– 2	Black (deep)	Loam	Canora
H– 3	Black (shallow)	Loam	Oxbow
H– 4	Black (deep)	Silty Clay Loam	Canora
H– 5	Degraded Black	Light Loam	——
H– 6	Black (shallow)	Light Loam	Blaine Lake
H– 7	Degraded Black	Silty Clay Loam	Tisdale
H– 8	Black (shallow)	Loam	Blaine Lake
H– 9	Degraded Black	Fine Sandy Loam	Shellbrook
H–10	Black	Light Loam	Yorkton
H–11	Grey (Podzol)	Loam	Waitville
H–12	Black (shallow)	Loam	Yorkton
H–13	Degraded Black	Fine Sandy Loam	Shellbrook
H–14	Grey (Podzol)	Sand	——
H–15	Black (deep)	Silty Clay Loam	Melfort

Description of Samples

Samples were selected from all four major soil zones as established by the Saskatchewan Soil Survey. These were chosen so as to obtain a wide variation in both clay and organic matter content. As may be seen in Table 2, the clay content varies from 1.6% in Jackpine Sand to 71.6% in Sceptre heavy clay. The organic matter content varies from 0.89% in Jackpine Sand to 11.7% in Melfort silty clay loam.

A short description of the various series types is given in Table 1.[3] The distribution of the samples in the various zones is shown in Figure 1.

The soils referred to in Table 1 may be placed in the following six groups on the basis of the parent material from which they were derived: (1) Sceptre, Regina, Indian Head, Melfort and Tisdale soils derived from heavy textured glacial lake deposits; (2) Fox Valley, Canora, and Blaine Lake derived from medium textured silty glacial lake deposits; (3) Haverhill, Weyburn, Oxbow and Waitville medium and light textured soils

[3] Saskatchewan Soil Survey Report No. 10.

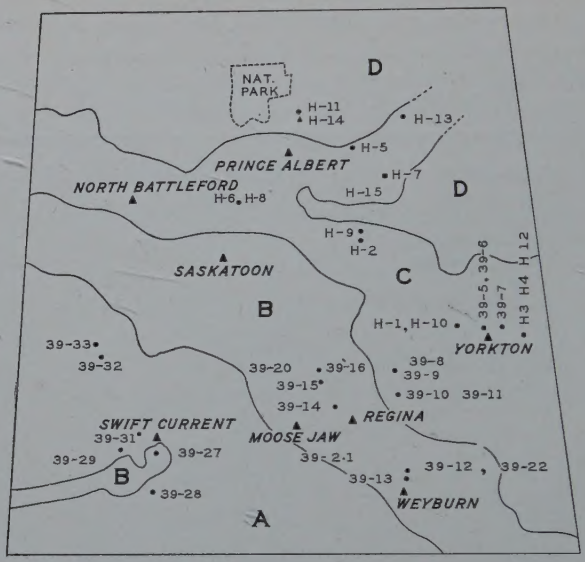

FIGURE 1. Sketch map of southern Saskatchewan, showing soil zones and approximate locations where samples were taken. A, brown soil zone; B, dark brown soil zone; C, black (park) soil zone; D, gray wooded zone. The numbers show locations of samples and are the same as sample numbers reported in Tables 1, 2, and 3.

developed on glacial till; (4) Yorkton medium and light textured soils developed on very calcareous, sandy clay and clay loam resorted till; (5) Cypress medium and light textured soils developed on mixed glacial till and tertiary sediments; (6) Hatton, Meota, Shellbrook and Jackpine Sands light textured soils developed on glacial lake and alluvial deposits.

DISCUSSION OF RESULTS

The study of soil moisture in its various states and activities is important from the standpoint of plant growth. Moisture relationships assume additional importance in areas of low rainfall. In such areas the study of moisture retention is of particular value in connection with the soil survey.

Laboratory studies of soil moisture may be divided into two classes; those within the hygroscopic range which give an indication of the colloidal properties of the soil, and those within and above the range of available moisture which are related in some degree to the state of aggregation as well as to mechanical and chemical constitution.

A good many soil moisture equilibrium points have come into use. Three of these, the Hygroscopic Coefficient, Wilting Coefficient, and Moisture Equivalent were selected for the purpose of studing Saskatchewan soil types with regard to moisture retention. Using these equilibrium points it was possible to study the soils selected at three quite different moisture contents.

Hygroscopic Coefficient

Robinson (17) states, "In natural soils, the water relationships are dominated by the presence of colloidal material." The hygroscopic

coefficient represents the amount of moisture that is adsorbed on the surface of the colloidal particles from an atmosphere of known relative humidity. It is an index of the surface activity of the soil. For the purpose of this study, it may be defined as the amount of moisture that a soil retains when in contact with an atmosphere of 45% relative humidity.

The results of the determination of the hygroscopic coefficient on 36 Saskatchewan soil types are shown in Table 2. In general as clay content decreases, the hygroscopic coefficient decreases. From Figure 2 it is apparent that the clay colloidal material is largely responsible for moisture retention at the hygroscopic coefficient.

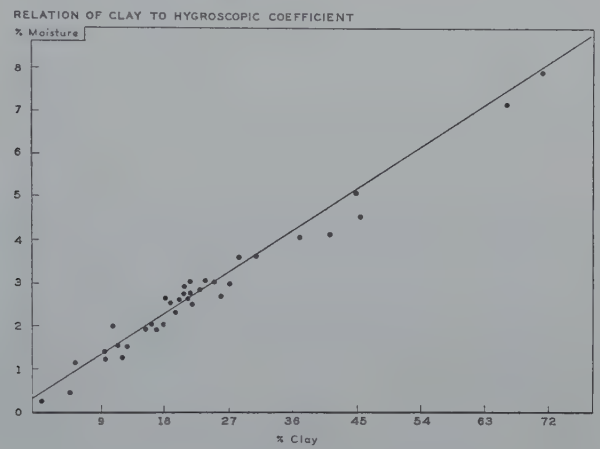

FIGURE 2. Relation of clay to hygroscopic coefficient.

The relationship between organic matter and hygroscopic coefficient is illustrated in Figure 3. The relationship is not as marked as that between clay and hygroscopic coefficient, but an examination of Table 2 indicates that samples with high organic matter content do show an increase in moisture content. For example, samples 39-9 (Indian Head Clay) and 39-8 (Indian Head Clay) differ by only 0.5% in clay content, but differ by 3.56% in organic matter content. There is a difference of 0.55% in the hygroscopic coefficient. Samples H-1 (Yorkton Light Loam) and 39-15 (Weyburn Loam) have approximately the same clay content but differ by 3.28% in organic matter. In this case there is a difference of 0.62% in the hygroscopic coefficient.

Thus it would appear that although clay content appears to be the dominant factor, organic matter also bears a relationship of some importance to the hygroscopic coefficient.

Statistical analysis of the data in Table 2 gives the following correlations:

(a) Correlation of clay and hyg coeff. 0.988

(b) Correlation of org. matter and hyg. coeff. 0.357

Correlation (a) is highly significant, whereas correlation (b) is not significant by Fisher's criteria (22).

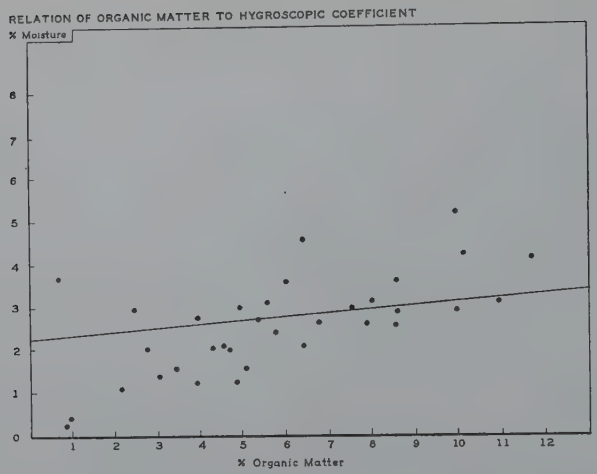

FIGURE 3. Relation of organic matter to hygroscopic coefficient.

TABLE 2.—MECHANICAL ANALYSIS, ORGANIC MATTER CONTENT AND EXPERIMENTAL AND CALCULATED HYGROSCOPIC COEFFICIENTS

Sample No.	Sand < 0.02	Silt 0.02 − 0.002	Clay < 0.002	Org. M.	Exp. Hyg. Coeff.	Calc. H.C. eqn. (1)	Diff.
39–32	8.6	16.4	71.8	3.76	7.80	7.72	+ 0.08
39–21	5.5	24.6	67.2	3.59	7.17	7.23	− 0.06
39– 9	28.1	20.7	45.4	6.42	4.55	5.10	− 0.55
39– 8	20.8	24.3	44.9	9.98	5.10	5.19	− 0.09
H– 7	24.1	25.6	41.4	10.10	4.22	4.83	− 0.61
H–15	31.6	23.1	37.1	11.70	4.12	4.46	− 0.34
39–10	41.4	22.8	31.5	6.00	3.62	3.65	− 0.03
H–4	20.9	42.3	28.9	8.55	3.58	3.49	+ 0.09
39–22	44.3	23.2	27.1	4.92	3.02	3.16	− 0.14
39–12	51.5	19.8	26.1	5.38	2.73	3.08	− 0.35
H–12	43.5	23.6	25.1	8.00	3.14	3.08	+ 0.06
39–20	42.1	24.1	24.1	5.60	3.06	2.87	+ 0.19
39–33	58.4	15.8	23.4	2.49	2.90	2.68	+ 0.22
39–11	41.0	26.3	22.4	10.90	3.06	2.92	+ 0.14
39–29	51.0	20.4	22.3	3.98	2.74	2.63	+ 0.11
39–13	48.2	22.3	22.2	9.88	2.86	2.85	+ 0.01
39–27	51.8	20.4	22.2	6.72	2.66	2.73	− 0.07
H–10	51.5	19.3	21.4	8.55	2.80	2.71	+ 0.09
H– 3	45.5	23.0	20.8	7.50	2.97	2.61	+ 0.36
H– 6	58.0	17.4	20.4	6.66	2.75	2.54	+ 0.21
H– 8	61.7	18.2	19.6	5.75	2.43	2.42	+ 0.01
H– 2	54.3	17.0	19.2	8.66	2.64	2.50	+ 0.14
H– 1	69.2	24.9	18.7	7.80	2.69	2.41	+ 0.28
39–15	58.0	19.6	18.1	4.52	2.07	2.21	− 0.14
39–28	64.3	16.0	17.4	2.70	2.03	2.07	− 0.04
39–14	63.8	15.5	16.8	4.58	2.05	2.08	− 0.03
39–16	65.9	12.5	16.2	4.31	2.02	2.01	+ 0.01
H– 5	67.2	15.1	12.9	5.13	1.51	1.71	− 0.20
39– 6	82.6	3.2	12.2	3.80	1.31	1.58	− 0.27
39–31	74.7	9.9	11.9	3.48	1.60	1.54	+ 0.08
39– 5	75.7	8.1	10.9	6.40	2.03	1.55	+ 0.48
H–13	77.9	9.8	9.9	3.10	1.41	1.31	+ 0.10
39–7	51.2	35.0	9.8	4.80	1.28	1.37	− 0.09
H– 9	84.0	7.1	5.1	2.15	1.18	0.79	+ 0.39
H–11	69.2	24.9	4.8	0.91	0.48	0.70	− 0.22
H–14	95.2	1.9	1.6	0.89	0.34	0.37	− 0.03

The regression equation for clay, organic matter and hygroscopic coefficient was calculated from this data and found to be:

$$\text{Eqn. (1).} \quad \text{Hyg. Coeff.} = (0.103 \times \text{clay}) + (0.04 \times \text{O.M.}) + 0.17$$

Using this equation, values of the hygroscopic coefficient were calculated for each of the 36 samples. These are shown in Table 2. There is a close agreement between the experimental and calculated values. With the exception of 7 samples they agree within 0.2%. The hygroscopic coefficient therefore appears to give a very good index of clay and organic matter and vice versa.

The clay content of each soil was calculated by using a modification of equation (1) given below.

$$\text{Eqn. (2).} \quad \%\ \text{clay} = \frac{\text{Hyg. Coeff.} - (0.04 \times \text{O.M.}) + 0.17}{0.103}$$

If the difference between the experimental and calculated values of the hygroscopic coefficient is divided by the regression coefficient for clay (0.103) the deviation between the experimental and calculated values for clay content is obtained. With the exception of 7 samples, the clay content may be estimated to within 2% of the values as obtained by mechanical analysis, since 0.1% difference between the observed and calculated values of the hygroscopic coefficient means approximately an error of 1% in estimating clay content.

Wilting Coefficient

The wilting coefficient has been defined as the weight of moisture present in the soil, when plants permanently wilt, expressed as a percentage of oven dry soil. It is an index of the amount of soil moisture unavailable to plants. This equilibrium point is important in studying irrigated soils, or soils of dry climates, where moisture is often the limiting factor in plant growth.

The wilting coefficients for the 36 soils are shown in Table 3. The samples are arranged in descending order of clay content. In general there is a decrease in moisture content at the wilting coefficient as the texture becomes lighter.

A comparison of samples 39-32 (Sceptre Heavy Clay), 39-29 (Fox Valley Loam) and 39-31 (Hatton Very Fine Sandy Loam) indicates this trend. These soils all have the same organic matter content but differ quite widely in the amount of moisture they retain at the permanent wilting point. Figure 4 gives a graphical illustration of the relation of clay content to the wilting coefficient. Although the agreement is not as close as with the hygroscopic coefficient, clay is still a very important factor.

While its effect is not as marked as the clay, organic matter is also quite important in moisture retention at the wilting coefficient. This is shown graphically in Figure 5.

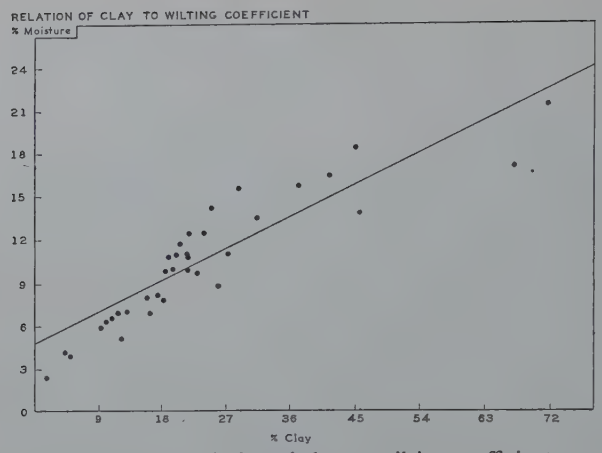

FIGURE 4. Relation of clay to wilting coefficient.

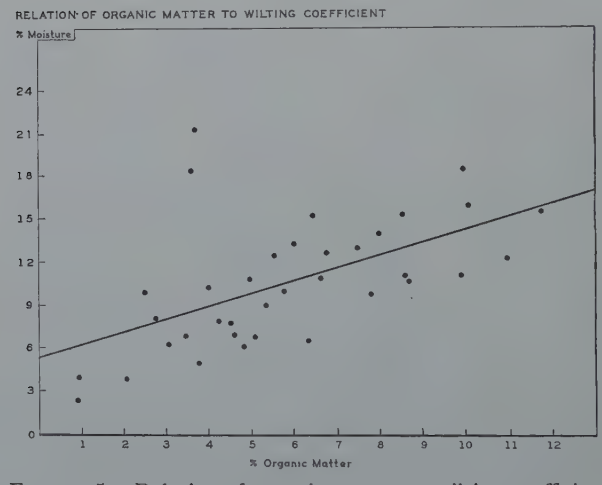

FIGURE 5. Relation of organic matter to wilting coefficient.

A comparison of samples 39-10 (Oxbow Clay Loam) and H-4 (Canora Silty Clay Loam), in Table 3, illustrates the effect of organic matter. H-4 has 2.4% less clay, but 2.5% more organic matter, than 39-10. This is reflected by an increase of 2.1% in the wilting coefficient. A number of other comparisons of this kind might be made, for instance, between samples 39-9 (Indian Head Clay) and 39-8 (Indian Head Clay), or 39-11 (Oxbow Loam) and 39-29 (Fox Valley Loam), or H-1 (Yorkton Light Loam) and 39-15 (Weyburn Loam). These comparisons indicate that soils of higher organic matter content generally have a higher moisture retention at the wilting coefficient.

Statistical analysis of the data in Table 3 give the following correlations:

(a) Correlation of clay and wilting coefficient 0.956
(b) Correlation of org. matter and wilting coefficient 0.758

Both of these correlations are significant according to Fisher's criteria (22).

TABLE 3.—CLAY AND ORGANIC MATTER CONTENT, EXPERIMENTAL AND CALCULATED WILTING COEFFICIENTS, AND MOISTURE EQUIVALENT

Lab. No.	Clay (0.002)	Org. M.	Exp. W. C.	Calc. W. C. eqn. (3)	Diff.	Calc. W. C. eqn. (4)	Diff.	Moisture equivalent
39–32	71.8	3.76	21.6	20.91	+ 0.69	23.60	− 2.00	43.9
39–21	67.2	3.59	19.6	19.53	− 0.07	22.08	− 2.48	39.7
39– 9	45.4	6.42	15.3	17.18	− 1.88	15.23	− 0.07	32.5
39– 8	44.9	9.98	20.0	19.63	+ 0.47	16.66	+ 3.34	41.6
H– 7	41.4	10.10	16.3	18.87	− 2.57	14.36	+ 1.94	33.4
H–15	37.1	11.70	15.7	19.20	− 3.50	14.10	+ 1.60	35.6
39–10	31.5	6.00	13.4	12.95	+ 0.45	12.80	+ 0.60	26.8
H–4	28.9	8.55	15.5	15.93	− 0.43	12.69	+ 2.81	35.1
39–22	27.1	4.92	10.7	10.94	− 0.24	11.23	− 0.53	22.3
39–12	26.1	5.30	9.0	11.00	− 2.00	10.47	− 1.47	24.1
H–12	25.1	8.00	14.1	13.10	− 1.00	11.55	+ 2.55	27.7
39–20	24.1	5.60	12.5	10.80	+ 1.70	11.40	+ 1.10	29.3
39–33	23.4	2.49	10.0	7.96	+ 2.04	10.92	− 0.92	22.1
39–11	22.4	10.90	12.3	14.91	− 2.61	11.40	+ 0.90	32.3
39–29	22.3	3.98	10.2	8.96	+ 1.24	10.50	− 0.30	23.1
39–13	22.2	9.88	11.2	13.99	− 1.79	10.81	+ 0.39	29.0
39–27	22.2	6.72	11.3	11.29	+ 0.01	10.29	+ 1.01	26.4
H–10	21.4	8.55	11.4	12.65	− 1.25	10.66	+ 0.74	26.5
H– 3	20.8	7.50	11.8	11.61	+ 0.19	11.10	+ 0.70	27.7
H– 6	20.4	6.66	10.9	10.79	− 0.11	10.53	+ 0.47	25.5
H– 8	19.6	5.75	10.0	9.82	+ 0.18	9.69	+ 0.31	23.2
H– 2	19.2	8.66	10.8	12.20	− 1.40	10.24	+ 0.56	19.5
H– 1	18.7	7.80	10.1	11.25	− 1.15	10.37	− 0.27	21.8
39–15	18.1	4.52	7.7	8.29	− 0.59	8.75	− 1.05	21.5
39–28	17.4	2.70	8.2	6.67	+ 1.53	8.64	− 0.44	19.2
39–14	16.8	4.58	7.1	8.14	− 1.04	8.68	− 1.58	17.6
39–16	16.2	4.31	8.15	7.74	+ 0.41	8.62	− 0.47	19.0
H–5	12.9	5.13	7.10	7.65	− 0.55	7.29	− 0.19	17.6
39– 6	12.2	3.80	5.25	6.34	− 1.09	6.77	− 1.52	14.0
39–31	11.9	3.48	6.95	5.99	+ 0.96	7.53	− 0.58	16.4
39– 5	10.9	6.40	6.60	8.24	− 1.64	8.65	− 2.05	13.2
H–13	9.9	3.10	6.25	5.17	+ 1.18	7.03	− 0.68	15.1
39–7	9.8	4.80	6.05	6.60	− 0.55	6.69	− 0.64	23.8
H– 9	5.1	2.15	4.15	3.18	+ 0.97	6.43	− 2.28	9.5
H–11	4.8	0.91	4.12	2.06	+ 2.06	4.60	− 0.48	17.7
H–14	1.6	0.89	2.50	1.25	+ 1.25	4 24	− 1.74	4.8

The following regression equation was derived relating clay, organic matter and the wilting coefficient.

Eqn. (3). Wilting Coeff. $= (0.245 \times \text{clay}) + (0.855 \times \text{O.M.}) - 0.1$

Using this equation, values of the wilting coefficient were calculated for all the samples. There is very good agreement between the experimental and calculated values as shown in Table 3. With the exception of H-7, H-15, and 39-11 the two values agree within 2%. This would indicate that the calculation of the wilting coefficient from clay and organic matter content is as reliable as the direct method, since in the latter case the limit of error varies between 1 and 2%.

Relation of Hygroscopic Coefficient to Wilting Coefficient

Since the hygroscopic coefficient and wilting coefficient both appear to vary directly with clay and organic matter content, it might be expected that a close mathematical relationship would be found between these two

equilibrium points. A correlation of 0.916 was found between the wilting coefficient and hygroscopic coefficient. The following equation was derived from the regressions.

Eqn. (4). Wilting Coeff. = (2.61 × Hyg. Coeff.) + 3.35

Values of the wilting coefficient were calculated from the experimental values of the hygroscopic coefficient using this equation. These are shown in Table 3. They agree very closely with the experimental values by the direct method. With the exception of samples 39-21, 39-8, H-4, H-12 and H-9, the differences are less than 2% which may be considered as within the limits of error by the direct method.

The correlations derived in the preceding sections, indicate a very close relationship between clay and organic matter, and moisture at the permanent wilting point and hygroscopic coefficient.

Moisture Equivalent

The determination of the moisture equivalent was included in this study to obtain some measure of the variation in moisture content at a point which falls within the range of available moisture. The results of the moisture equivalent determinations on the 36 soils studied (Table 3) show that there is a general decrease in moisture equivalent as clay content decreases. At the same clay content higher organic matter appears to increase the moisture equivalent. However, the results are quite erratic.

Attempts were made to establish some definite relationship between mechanical composition and the moisture equivalent, and between moisture equivalent and either of the other two equilibrium points studied. The variations between the observed and calculated results were so great that there did not appear to be much value in relationships as found from these data. The inconsistency of results may be partly due to variations in organic matter content, more especially because of the effect that organic matter may have on the state of aggregation. According to Briggs and McLane (5) and Fisher (9) the moisture retained at the moisture equivalent is that held in the smaller pores of the soil. Any factor which might affect a change in the percentage of small pores capable of retaining moisture against a force equivalent to 1000 times gravity would therefore apparently influence the value of the moisture equivalent obtained.

However, Alway and McDole (2) and Middleton (12) state that the moisture equivalent is a valuable indication of texture. Veihmeyer and Hendrickson (20) also found that for medium textured soils, the moisture equivalent gives a good index of the normal field capacity.

SUMMARY

The Hygroscopic Coefficient, Wilting Coefficient and Moisture Equivalent were determined on 36 Saskatchewan soil types. The relationship of soil composition to moisture retention at these equilibrium points was investigated.

The necessity of considering organic matter, as well as clay content in such studies was illustrated in the results obtained.

The hygroscopic coefficient was found to be closely related to the content of clay and organic matter in the soil, but particularly to the former. The following relationships were established.

Eqn. (1) Hyg. Coeff. = (0.103 × clay) + (0.04 × O.M) + 0.17

$$\text{Eqn. (2) \% clay (0.002 mm.)} = \frac{\text{Hyg. Coeff.} - (0.04 \times \text{O.M}) + 0.17}{0.103}$$

The hygroscopic coefficient can be used in calculating the clay content of a soil providing the organic matter content is known.

A correlation was obtained between clay and organic matter content, and the Wilting Coefficient, and also between the Hygroscopic Coefficient and the Wilting Coefficient.

The following equations for calculating the wilting coefficient were established:

Eqn. (3) Wilting Coeff. = (0.245 × clay) + (0.855 × O.M) − 0.1

Eqn. (4) Wilting Coeff. = (2.61 × Hyg. Coeff.) + 3.35

The relation of moisture equivalent to mechanical composition, hygroscopic coefficient and wilting coefficient was also studied. No close relationship was found, either between soil composition and moisture equivalent or between moisture equivalent and the other soil moisture equilibrium points.

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THE IMPORTANCE OF VARIOUS ROOTS TO THE WHEAT PLANT[1]

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A knowledge of the relative values of the various roots to the wheat plant is useful in evaluating the effect of rootrot diseases on the ultimate yields. Such a disease as "browning" rootrot of wheat, in which the killing of the growing points of the crown roots is a conspicuous symptom, is one in which such a knowledge may serve to clarify conceptions of the disease. It has been shown (4) that a much less evident effect of the disease is the killing of the lateral rootlets produced on the seminal roots at depths down to 20 inches. Papers by Simmonds and Sallans (5, 6) have supplied some information with regard to the relative importance of the seminal and crown root systems as determined by root amputation experiments. The present paper is an extension of this work under more normal crop conditions.

METHODS

The relative contributions of the various root classes to yield were determined using the amputation method. The crop was sown in rows 6 inches apart, as is the usual practice with cereal crops. The treated plots consisted of single rows 3 feet apart. The intervening 5 rows of untreated plants supplied normal field competition. Following the root-cutting operation the untreated plants that had been crushed down recovered in a few days to show little evidence of injury. As in previous work (5, 6) the amputations were made after the plants had become well established and had developed a few crown roots.

The roots amputated were the true seminal roots, including the primary root, the first and second pair of lateral roots, the sixth seminal root and those roots to which McCall (1) has applied the term coleoptile axillary roots. These may be one, two or three in number and have sometimes been classed as seminal roots, Percival (2, page 35), though they appear to be adventitious.

Data were treated statistically by the analysis of variance method as used by Yates (10) for factorial experiments.

PRELIMINARY WORK

In 1934 a number (402) of mature plants grown in rows 6 inches apart and 1 rod long, were carefully removed from the soil after cutting the roots about 2 inches below seed level with a spade. The plants were classified into groups depending on the number of seminal roots, including the coleoptile axillary roots. No account was taken of the crown roots. In 1935 there were 150 plants similarly classified. The data, including

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the number of heads, the yield of grain, the number of kernels on a single plant basis, and disease ratings for 1934, are given in Table 1. The disease ratings are based on subcrown internode lesions, and are calculated by the method set forth by Russell and Sallans (9).

TABLE 1.—EFFECTS OF NUMBER OF SEMINAL ROOTS UPON YIELD OF GRAIN

Year	No. of seminal roots per plant	No. of plants	No. of heads per plant	Yield of grain per plant	No. of kernels per plant	Weight per kernel	Disease rating
				gm.		mg.	
1934	3	27	2.48	1.167	40.2	29.0	15.9
	4	51	2.57	1.236	41.3	29.9	13.5
	5	116	3.15	1.531	52.5	29.2	11.5
	6	122	3.35	1.669	56.6	29.5	11.1
	7	86	3.57	1.848	62.0	29.8	11.0
1935*	4	8	4.00	1.177	67.7	17.3	—
	5	21	4.09	1.503	80.7	18.6	—
	6	64	4.56	1.676	92.0	18.2	—
	7	50	5.04	1.830	101.4	18.1	—
	8	7	5.57	1.978	110.3	17.9	—

* Considerable damage was done by stem rust which reduced the weight per kernel.

In both years there is evident an influence of the number of seminal roots on yield of grain per plant. The increase in yield co-incident with an increase of one in the number of seminal roots cannot, however, be taken as a measure of the contribution of that root directly to yield. There is a positive correlation usually present between the number of seminal roots and the number of crown roots formed. Thus the increases in yield with increasing numbers of seminal roots are partly due to contributions by the crown roots.

The data of Table 1 indicate that the increase in yield with more numerous seminal roots is brought about by the production of more kernels per plant rather than by heavier kernels. In 1934 the disease ratings were highest on plants with few seminal roots. There is a suggestion here that some seminal roots had been prevented from developing by infections of rootrotting organisms. However, this probably is not the case. Ordinarily in Saskatchewan the seminal root system of wheat is well established before subcrown internode lesions become common. It may be suggested that the smaller, weaker plants with fewer seminal roots are more susceptible to infection by common rootrot fungi than are the larger stronger plants possessing more seminal roots.

To obtain more accurate and detailed information on the contribution of various classes of roots to yield, and the effect of injury to roots on yield the amputation method (5) was used.

AMPUTATION EXPERIMENTS

The experiments were based on a $2 \times 2 \times 2$ factorial design with the second order interaction confounded, as described by Yates (10). The amputations were of three types corresponding to the three factors of the

design, namely: *(a)* amputation of the primary seminal roots; *(b)* amputation of the first pair of lateral seminal roots, and *(c)* amputation of the second pair of lateral seminal, the sixth seminal and the coleoptile axillary roots. These will be designated respectively, *a*, *b*, and *c*, and the crown roots as *d* for convenience. All possible combinations of presence or absence of each type of amputation may be represented as follows:— O, *a*, *b*, *ab*, *c*, *ac*, *bc*, and *abc*. The eight treatments were arranged in two half-blocks of four plots each in such a way that the variance for the second order interaction *abc* was identified with the variance of blocks. The treatments were replicated four times with the usual randomization of plots within half-blocks and of half-blocks within blocks. Each plot consisted of a single row, 10 feet long.

Two experiments were laid down on this plan, the first at Indian Head in 1938 using Marquis, and the second at Saskatoon in 1939 using Renown wheat. At the time the plants were operated upon, 100 representative plants were taken up and their development recorded. These data are given in Table 2.

TABLE 2.—COMPARATIVE DEVELOPMENT OF PLANTS WHEN AMPUTATIONS WERE MADE IN 1938 AND 1939

	1938	1939
Dates of amputation	June 22–24	June 30 – July 4
Height	35 cm.	61 cm.
Stage of development	5th to 6th leaf	76% headed
No. of tillers	4.7	4.3
No. primary roots	0.97	0.92
No. first pair lateral roots	1.96	1.95
No. second pair lateral roots	0.55	1.44
No. of sixth seminal roots	0.04	0.24
No. of coleoptile axillary roots	1.45	1.31
No. of crown roots	7.5	24.2

In 1938 weather conditions following the amputations were mostly favourable for recovery of the plants from initial wilting. However, one or two hot days resulted in the drying up of a few lower leaves and the death of a few plants, especially where the seminal roots were all cut off. In 1939 conditions were very favourable for the recovery of the injured plants, only the more severely treated of which showed some wilting during the first day or two.

Data taken at harvest time included counts on the numbers of roots of the various classes, tillers per plant, number of plants per row, and yield. The data, on a single plant basis, are summarized in Table 3.

The data of Table 3 are based on four replications. However, the design of the experiment is such that further replications are present. For example, of the 32 plots comprising an experiment 16 received the treatment *a* (amputation of the primary root), while 16 did not. The effect of treatment *a* is, therefore, measured by comparing the two sets of 16 plots each. This is done in the presence and absence of the various other treatments, so that interaction effects are measured. The data of

TABLE 3.—NUMBER OF HEADS AND ROOTS OF THE VARIOUS CLASSES PER PLANT AT MATURITY, WEIGHT OF DRY TOPS IN 1938 AND YIELDS OF GRAIN IN 1939

Place and year	Amputation treatment*	Number of heads	Number of seminal roots					Number of crown roots	Yields
			Primary	First pair lateral	Second pair lateral	Sixth	Coleoptile axillary		
Indian Head 1938	Control	3.54	0.99	1.97	0.50	0.05	1.34	20.2	4.135
	a	2.95	0.16	1.75	0.53	0.04	1.35	18.2	3.555
	b	2.84	0.95	0.25	0.29	0.02	1.09	18.3	3.602
	ab	1.95	0.07	0.25	0.39	0.03	1.08	14.5	2.100
	c	2.95	0.95	1.73	0.15	0.01	0.33	18.2	3.792
	ac	2.65	0.10	1.73	0.11	0.00	0.48	16.0	3.165
	bc	2.51	0.90	0.13	0.06	0.00	0.46	16.2	2.860
	abc	1.41	0.02	0.20	0.06	0.00	0.46	11.0	1.535
Saskatoon 1939	Control	3.89	0.95	1.86	1.51	0.20	1.16	21.6	2.075
	a	2.32	0.27	1.50	1.27	0.22	1.06	19.5	1.605
	b	2.77	0.76	0.40	0.95	0.16	0.96	21.9	1.855
	ab	2.28	0.18	0.47	0.88	0.12	0.87	22.7	1.635
	c	2.97	0.92	1.52	0.43	0.11	0.61	22.5	1.860
	ac	2.29	0.16	1.42	0.51	0.08	0.51	19.8	1.745
	bc	2.48	0.77	0.41	0.27	0.05	0.32	21.3	1.790
	abc	1.98	0.08	0.20	0.20	0.03	0.18	22.4	1.220

* *a*—represents amputation of primary seminal root.
b—represents amputation of first pair of lateral seminal roots.
c—represents amputation of second pair lateral, sixth seminal roots and coleoptile axillary roots.

TABLE 4.—MAIN AND INTERACTION EFFECTS OF AMPUTATIONS UPON NUMBERS OF HEADS AND ROOTS, AND UPON YIELDS, AND THEIR SIGNIFICANCE AS DETERMINED BY ANALYSES OF VARIANCE

Place and year	Main and interaction effects*	Number of heads	Number of seminal roots			Number of crown roots	Yields
			Primary	First pair laterals	Second pair laterals		
							grams
Indian Head 1938	Total	20.80	4.14	8.01	8.83	132.6	24.73
	A	−2.88	−3.44	−0.15	0.23	−13.2	−4.03
	B	−3.38	−0.26	−6.35	−0.95	−12.6	−4.53
	AB	−1.10	−0.08	0.29	−0.03	− 4.8	−1.61
	C	−1.76	−0.20	−0.43	−4.61	− 9.8	−2.03
	AC	.08	−0.02	0.29	−0.01	− 1.6	−0.13
	BC	.02	0.00	0.09	0.89	− 1.4	−0.57
	5% signif. level	0.77	0.14	0.18	0.12	5.2	1.26
	1% signif. level	1.06	0.19	0.24	0.16	7.1	1.73
Saskatoon 1939	Total	19.98	4.09	7.78	12.66	171.7	13.785
	A	−2.24	−2.71	−0.60	−0.80	−2.9	−1.375
	B	−0.96	−0.51	−4.82	−2.68	4.9	−0.785
	AB	0.26	0.17	0.32	−0.06	6.7	−0.205
	C	−0.54	−0.23	−0.68	−6.06	0.3	−0.555
	AC	−0.12	−0.19	−0.02	0.24	−0.3	0.005
	BC	0.10	0.05	0.16	0.28	−2.1	−0.405
	5% signif. level	0.94	0.14	0.19	0.30	5.3	0.561
	1% signif. level	1.28	0.20	0.26	0.42	7.2	0.768

* The second order interaction was confounded in both experiments.
N.B. To find the effect of a treatment on a single plant basis, the data in this table are divided by 4 since they are the totals of four comparisons between pairs of treatments as given in Table 3.

Table 3 are presented in Table 4 with the various main effects and interactions sorted out by the method given by Yates (10). The data for second pair lateral, sixth seminal root and coleoptile axillary roots are combined.

It is evident that the main effects of root amputation on yield are significant, the effect being to reduce the yields. The interactions, however, produced no significant effect upon yield. It may be concluded, therefore, that injury to the various classes of roots produces effects upon the plants, as reflected in yield, which are independent of each other. Rather more of the interaction effects on the numbers of roots of the various classes, at harvest time, reached the level of significance than would be expected on the basis of chance. This probably is because of a tendency to injure nearby roots in making amputations. Thus in cutting the primary root one of the other seminals might easily be broken.

The signs of the main effects of root amputations on yield are minuses indicating reductions in yield. The effect (A) of cutting off the primary root reduced the yield per plant by $1.375 \div 4 = 0.344$ grams. Similarly the amputation of the first pair of lateral seminals reduced the yield by 0.196 grams and loss of roots of class c (second pair of lateral seminal, sixth seminal and coleoptile axillary) reduced the yield by 0.139 grams. Since loss of roots of these three classes caused the above mentioned losses in yield, it may be assumed that if they had not been amputated they would have contributed like amounts to the plant yields.

It is evident from the counts of roots at harvest that many roots were missed in amputations and many accidentally broken due to difficulties in technique. The above estimates of reduction in yield are, therefore, in error. It is possible to set up four equations for each experiment from the data in Table 4, from which the contributions of individual roots of the various classes may be calculated. To do this two assumptions must be made: I. That each individual root makes a definite contribution toward the final yield independent of other roots; II. That a portion of the error of determination of yield is removed from the equations, in the interactions, which are non-significant, at least in the yield data. From the total effects in the 1939 experiment it may be seen that 4.09 primary roots (A) plus 7.78 first pair lateral roots (B) plus 12.66 of the remaining classes of seminal roots (C) plus 171.7 crown roots (D) produced a yield of 13.785 grams of seed. Similarly reductions of 2.71, 0.60, 0.80 and 2.9 of the various classes of roots, caused in making the primary root amputations, reduced the yield by 1.375 grams. Using the symbols A, B, C, D, as above for the various classes of roots the following equations may be written down from Table 4.

$$\begin{aligned}
4.09A + 7.78B + 12.66C + 171.7D &= 13.785 \\
2.71A + 0.60B + 0.80C + 2.9D &= 1.375 \\
0.51A + 4.82B + 2.68C - 4.9D &= 0.785 \\
0.23A + 0.68B + 6.06C - 0.3D &= 0.555
\end{aligned}$$

On solving for the unknowns, A is found to equal 0.3925, B equals 0.1466, C equals 0.0631, and D equals 0.0596. Similarly from the 1938 data the values are, $A = 0.6351$, $B = 0.4036$, $C = 0.0846$ and $D = 0.1367$.

Using these data and the data as to root numbers, Table 3, the expected yields of grain may be calculated. These expected yields are compared with the actual yields in Table 5.

TABLE 5.—ACTUAL AND EXPECTED YIELDS

	Amputation treatments	Dry top weights, 1938			Yields of grain, 1939		
		Actual	Expected	Deviations	Actual	Expected	Deviations
0	None	4.135	4.360	0.225	2.075	2.114	0.039
a	Primary root	3.555	3.460	−0.095	1.605	1.650	0.045
b	First pair laterals	3.602	3.314	−0.288	1.855	1.795	−0.060
ab	Primary and 1st pair laterals	2.100	2.248	0.148	1.635	1.612	−0.023
c	2nd pair laterals, etc.	3.792	3.831	0.039	1.860	1.999	0.139
ac	Primary and 2nd pair laterals	3.165	2.999	−0.166	1.745	1.522	−0.223
bc	1st and 2nd pair laterals	2.860	2.877	0.017	1.790	1.672	−0.118
abc	All seminal roots	1.535	1.647	0.112	1.220	1.423	0.203

The deviations of the expected yields from the actual are traceable directly to the interaction effects in root numbers, Table 4. These, as pointed out before, are ascribable to error and to spurious effects caused by accidental injuries.

DISCUSSION

The amputation of various roots of the wheat plant growing in the field has proven a useful method of determining the relative values of roots to the plant in terms of yields. It appears that when the various roots are well established they contribute independently of each other to the water and mineral supply of the plant as indicated in final yield of grain.

The individual root which contributes most to the final yield is the primary seminal root, which is also the first root to appear, while the first pair of lateral seminal roots is of about equal importance. These three roots are almost invariably produced by seedlings growing under normal conditions. They are responsible for obtaining most of the water and soil nutrients from the time of emergence of the seedling till the first crown roots are well established, a period of at least six weeks under Saskatchewan conditions. From the data in this and previous papers it is evident that after the establishment of the crown roots these three seminal roots continue to make important contributions to the growth of the plant. The primary root was shown to contribute about 0.39 grams to the final yield of the plant in the 1939 experiment between the time of amputation, when the crown roots were all established, and maturity of the plant. At the same time each root of the first seminal pair contributed about 0.15 grams of grain.

The second pair of seminals, the sixth seminal and coleoptile axillary roots proved much less useful to the plant than the first three seminal roots. However, they appeared to contribute about as much per root as did the crown roots in 1939, though less in 1938. Of this group the sixth seminal is usually not developed and when present is usually short and of small diameter. The number of the second pair laterals appears to be influenced by conditions. There were two and a half times as many roots of this

class per plant at Saskatoon in 1939 as at Indian Head in 1938. It would appear to be of considerable advantage to the plant to have these roots well developed, as they become established early in the life of the seedling and probably continue their usefulness till shortly before maturity. McCall (1) has shown that the two roots of the second lateral pair and the sixth seminal root are much less favourably situated with reference to the endosperm than are the first three roots to be formed. Hence they are much less well developed and, consequently, less important to the plant. The coleoptile axillary roots are of considerable importance. While the possible number of roots of this class is three according to McCall (1), the writer has found them to be usually either one or two to a plant though sometimes they are absent. Except that they appear much earlier they might justifiably be classed with the crown roots.

The contribution of the crown roots to final yield of grain is probably much more variable under the semi-arid growing conditions of Western Canada than that of the other three classifications of roots made in this paper. At Saskatoon in 1939 the crown roots individually were less valuable than roots of the other classes taken singly. In the aggregate, however, they contributed about 61% of the total yield. At Indian Head in 1938 crown roots contributed about 63.6% of the total yield. In 1929 at Indian Head (5) plants with the seminal roots cut off before the middle of June and supported only by the crown roots produced 65.5% of the check yield. In 1930 (6) similarly treated plants produced only 36% as much grain.

The earlier the amputation of the seminal roots the greater is the reduction in final yield, as was shown by Simmonds and Sallans (6). In their work, amputations, made as late as July 21, caused no loss in yield, whereas when they were made on June 9th, the loss amounted to 64% of the yield as shown in the controls. It would appear that still earlier amputation of the seminal roots, before the appearance of the crown roots is likely to cause the death of the seedling, especially if the weather is hot and dry. On the other hand if only one or two seminal roots are amputated or injured in early stages of development, it is probable that the remaining seminal roots may make increased growth, making up in part for the loss of roots. Likewise early injury to the seminal roots may result in an early appearance of crown roots. Russell (8) has found this to be true of wheat in which the seminal roots were almost entirely destroyed by *Ophiobolus graminis* Sacc.

While the results of amputating roots have been measured in terms of yield, the physiological effect of amputation is to remove portions of the absorptive area of the roots with consequent reduction in water and nutrients supplied to the plant from the soil. Figure 1 represents the relative amounts of water supplied by the seminal and crown root systems at any time during the life of the wheat plant. The form of the upper curve, showing the total amount of water supplied was drawn from data obtained by the author in transpiration studies (3). The first part of the lower curve, representing water supplied by the seminal roots is identical with the upper curve to the point where the crown roots began to appear. The rest of the curve is interpolated, its form being made to correspond roughly with the upper curve. The cross-hatched area bears the same relation to the

area representing all water supplied by the two root systems after seminal root amputation that the loss in yield of plants with seminal root amputations bore to the yield of the controls in the 1939 experiment. No attempt was made to show short term fluctuations but only the broad trends in water consumption.

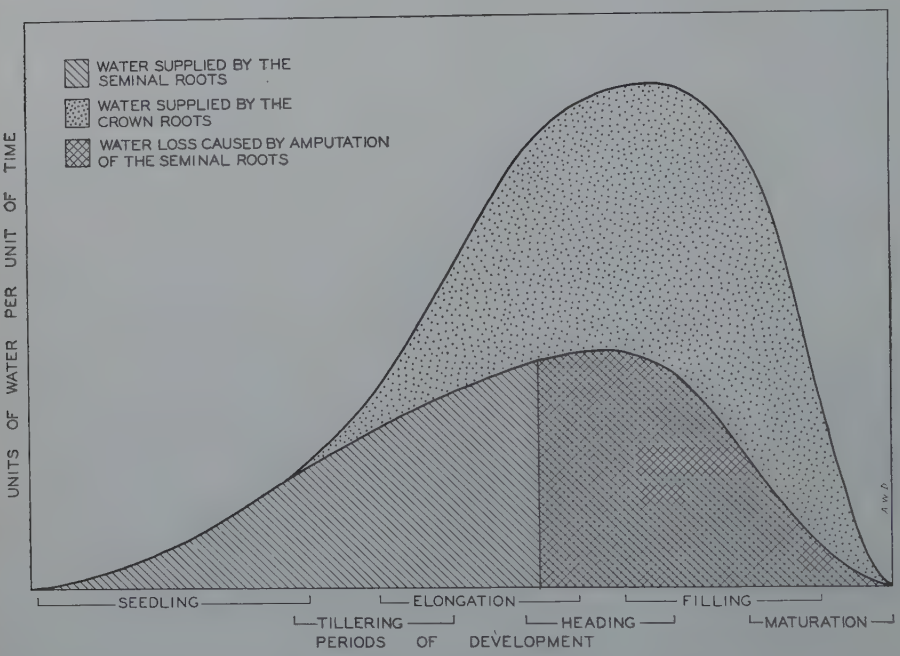

FIGURE 1. Curves representing the relative amounts of water supplied by the seminal and crown root systems of wheat plants at successive periods of development from the seedling stage to maturity. The lower curve represents water supplied by the seminal roots. The cross-hatched area below this curve represents water which is lost to the plant following amputation of the seminal roots, represented by perpendicular line.

During the first 6 weeks, approximately, all the water is provided by the seminal roots. The crown roots are then developed and take up an increasing part in meeting the water requirements of the plant, as this need increases with the transpiring area of the plant and the rising temperature of the environment. If the seminal roots are amputated early in July, as was done in the 1939 experiment, then a portion of the water supply of the plant, drawn largely from soil 1 to 4 feet below the ground level, is cut off, as shown in the shaded area of Figure 1. At this time the plant is still laying down structural materials in new tillers, rootlets, floral structures and lengthening culms. Consequently, the reduced water supply may result in a lessened growth rate and a smaller plant. At the time of flowering the reduction in roots and water supply brings about a downward adjustment in the number of fertile spikelets and flowers per spikelet. After flowering when photosynthetic products are largely concentrated in the kernels and growth has stopped, except perhaps in the rootlets, the reduced supply of water and soil nutrients results in a smaller yield than in the controls.

The curves of Figure 1 will vary with soil moisture conditions over a very wide range. If the reserve of water in the soil at the beginning of the season is just sufficient to bring the seedlings on to the stage of crown root development and rainfall is sufficient to keep the upper foot or so of soil moist enough for plant growth then the contribution of water by the seminal roots will be very small. If on the other hand there is a fair supply of water in the soil and rainfall is very light, the plants may be supported almost entirely by the seminal roots and the crown roots have little influence on the final yield. This condition was found in previous work (7).

The relation of the finding in this paper to the influence of rootrots on final yields seems at first glance fairly obvious. Browning rootrot of wheat often causes an amputation of crown roots, by rotting the growing tip or a segment of the root behind the tip. There is thus a loss in number of crown roots and, judging by the findings in this paper, there is a corresponding loss in final yield. The higher the percentage loss of crown roots the greater will be the loss in yield, especially under conditions when crown roots in the aggregate contribute significantly to yield. Browning usually becomes evident in the plants after the seminal roots are well established. While the main axes of the seminal roots usually escape being rotted off, the lateral branches have been shown (4) to suffer severe rotting, so that the efficiency of the seminal roots is greatly curtailed. The action of the fungus here is amputation of the rootlets rather than the main roots but the effect is similar in that the effective absorptive surface is greatly reduced. An important difference from complete amputation of the main axes of the roots is that the growing points remain active. The roots continue to grow downward into layers of soil where the fungus is relatively or completely absent. In this way the plants are able to show signs of recovery, though retarded, after initial symptoms of the disease.

In the case of the rootrot known as take-all varying degrees of amputation of roots may occur. The results in the host plants vary from little reduction to large reductions in yield, or even death of the plant. Simmonds, Russell and Sallans (4) have shown cases of complete destruction of seminal roots by take-all in plants which continued a meagre growth on a few crown roots.

Common rootrot (*Helminthosporium sativum* and certain *Fusarium* spp.) does not ordinarily result in effective amputation of roots, in Saskatchewan. There may be some reduction in numbers of lateral branches, and in number of seminal roots, especially if the disease was seed-borne. There is nevertheless considerable stunting and reduction in yield in infected plants. The disease results in reduced vigour of growth. If the onset of disease is prior to crown root formation the number of such roots may be considerably reduced as a result of this lack of vigour in the plant, and to some extent by direct invasion of crown roots and the tissues giving rise to crown roots.

The parallel between artificial root amputation and the rotting of certain root tissues by root pathogens seems to be close. The method of root amputation as a means of evaluating yield losses by rootrots appears well established.

SUMMARY

The relative importance of the seminal and crown root systems and of individual roots to the wheat plant were studied by means of root amputations and their effects upon plant yields. Roots after becoming well established were found to contribute independently of each other to the final yield of grain. The primary seminal root proved the most valuable individual root being equally as important as both roots of the first pair of lateral seminal roots. The second pair of seminal roots, the sixth seminal and coleoptile axillary roots proved much less valuable than the first three seminal roots to appear. They were about as valuable root for root as the crown roots.

The contributions of the crown roots while individually small, were found in the aggregate usually to be larger than those of the seminal roots. Much variation in crown root contributions to yield may occur with variation in seasonal precipitation and soil moisture.

The relation of the roots to the plant's water supply and the influence of root amputation upon water supply is discussed, as is also the parallel between artificial amputation of roots and that brought about by root infection by various rootrotting soil micro-organisms.

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THE EXAMINATION OF CEREAL SEEDS FOR DISEASE AND STUDIES ON EMBRYO EXPOSURE IN WHEAT[1]

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Summaries of tests conducted on samples of cereal seed of the 1940 crop, submitted by the Plant Products Division, Saskatoon, for pathological examination, are presented. The routine laboratory tests were done by all members of the staff. In addition, special studies, as outlined below, were conducted.

The number of samples received and their classification is given in Table 1.

Table 1.—Samples of wheat, oats and barley seed examined for disease in 1941

	Classes				
	Foundation	Elite	Registered	Certified	Total
Wheat	6	32	65	469	572
Oats	2	28	119	27	176
Barley	1	8	46	14	69

The routine laboratory tests which were employed included: (1) visual examination, (2) examination for exposed embryos, (3) centrifuge test, (4) incubation test. These tests were described previously (4, 5). In a few cases the sample was too small to permit all tests being done.

LABORATORY EXAMINATION OF 1940 SAMPLES

The data for wheat are given in Table 2, arranged according to soil zones and varieties. A description of the soil zones was given by Russell and Ledingham (4). The results for 1940, as given in Table 2 agree in most respects with those for 1939 (4). The amount of smudge increased from zone 1 to zone 3, but decreased in zone 4 in 1940. The same was true of the number of kernels producing *Helminthosporium sativum* Pammel, King and Bakke in the incubation test. There was, however, no strict relationship between smudge as recorded in Table 2, and *H. sativum*. It was shown later that a certain type, which may be called severe smudge, is caused by *H. sativum*. This type occurs much less frequently than the ordinary mild type, which appears to be prevalent in some of the newer varieties. The cause of the latter is not known. Piebald was most prevalent in the grey soil zone, as in other years. This is a nutritional trouble. As in 1939, embryo exposure was most severe in Regent, Renown

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TABLE 2.—SUMMARY OF THE RESULTS OF LABORATORY TESTS ON WHEAT ARRANGED ACCORDING TO SOIL ZONES AND VARIETY

Soil zone or variety	Total samples	Visual examination				Embryo exposure	Microscopic examination			
		Smudge		Piebald			Bunt		*H. sativum*	
		Samples	Kernels	Samples	Kernels	Kernels	Samples	Spore load	Samples	Kernels
	No.	%	%	%	%	%	%	No.*	%	%
Zone										
1. Brown	75	39.0	0.49	16.0	0.71	32.9	17.3	15	24.7	0.34
2. Dark brown	130	40.0	0.57	13.3	0.17	37.1	9.0	6	32.3	0.53
3. Black	306	47.4	0.90	11.1	0.91	29.1	4.9	3	37.5	0.72
4. Grey	51	31.4	0.25	27.5	6.25	28.1	13.7	9	17.7	0.37
Variety										
Regent	340	37.4	0.26	11.7	1.59	32.4	15.1	7	34.9	0.61
Apex	91	50.5	0.95	14.1	0.94	29.5	10.6	7	27.1	0.48
Thatcher	67	48.0	1.79	13.6	0.70	18.3	5.1	3	23.7	0.42
Renown	30	30.0	0.15	14.3	0.17	31.3	10.0	6	43.0	1.10
Marquis	15	53.5	0.27	40.0	1.20	23.2	6.7	4	27.0	0.60

*Approximate number of spores per kernel.

and Apex and least severe in Thatcher. An explanation of this is given in another section of this paper. The amount of bunt smut occurring in the samples was small. It was present on 8·3% of the samples which had an average spore load of less than 10 spores per kernel. Ordinarily under Saskatchewan conditions, average spore loads of about 120 spores per kernel for wheat and 200 for oats and barley produce from 0·1 to 1·0% of smutted heads in the crop. An infection of 5% or over in the field, in the case of wheat, would indicate a likelihood of the threshed grain being graded "smutty" according to information available.

Similar tests were conducted on 165 samples of oats, including the varieties Vanguard, Victory, Banner and Gopher. An average spore load of about 500 spores of smut per kernel was found on 66% of the samples of Vanguard. The results for the other varieties were as follows: Victory, 180 spores on 82%; Banner, 60 spores on 50%, Gopher, 80 spores on 75%. *Alternaria* was present on all samples and several were infected with *Rhizopus* and *Penicillium*. It is interesting to note that *H. sativum* was fairly common on the oat kernels when they were incubated. This fungus does not commonly attack the oat plant. Spores of *Fusarium* were seen infrequently in centrifuged washings.

The varieties O.A.C. 21, Rex, Plush, Regal, Prospect and Olli were represented in the 63 samples of barley tested. An average spore load of about 75 spores of smut per kernel was found on 25% of the samples in some varieties and 60% in others, Regal and O.A.C. 21 having the most. *H. sativum* developed on 18 of the samples and on less than 1% of the kernels. The greatest occurrence of this fungus was 1% on O.A.C. 21. *H. sativum* will cause rootrot of barley if a virulent strain is present on

the seed. It is frequently the cause of smudge in barley seed. This discolouration was present in 40 of the samples, the average occurrence being 1·5%.

EMBRYO EXPOSURE IN WHEAT SEED

In an earlier report by Russell and Ledingham (4) it was shown that embryo exposure may be of considerable importance. This confirmed observations made several years ago by the senior author when called upon to investigate a serious wheat seed problem.

There are two general types of fractures which occur in the protective layers of the wheat embryo. In one, the covering layers over the tip of the embryo, the pericarp and seed coats, are chipped or broken off, in the other, the injury is manifested by longitudinal cracks through the pericarp and seed coats. The embryo is exposed in both cases, Figure 1.

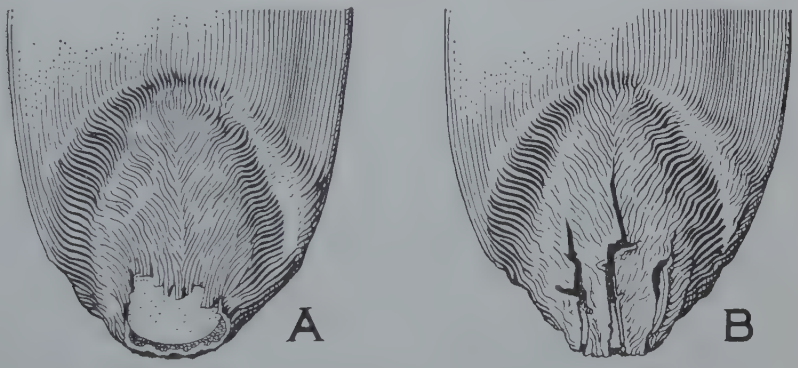

FIGURE 1. Two types of embryo exposure. (A) Pericarp and adjacent layers chipped off exposing the tip of the coleorhiza and (B) fractures in the pericarp which covers the face of the embryo.

Varietal Differences

Most of the ordinary wheat varieties grown in Western Canada were compared in respect to the length of the embryo projection. This work

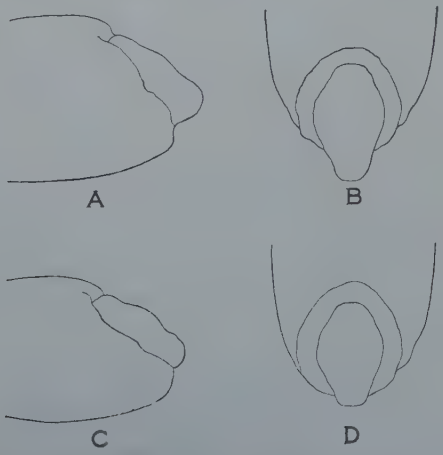

FIGURE 2. A comparison of a long embryo tip as shown in kernels of Regent wheat, (A) and (B) and a short embryo tip in kernels of Canus, (C), and (D).

was done by Miss E. M. Child. The embryo projection is that portion of the embryo tip with protective layers which extends beyond the attachment scar (the point where the kernel had been attached to the rachilla), Figure 2. Measurements were made from the scar to the tip of the projection. A binocular was used and the measuring units were the divisions of an ocular micrometer. The data are recorded in Table 3 and were analysed by the analysis of variance method by Dr. B. J. Sallans, on the basis of four replications.

TABLE 3.—DISTRIBUTION OF ONE HUNDRED KERNELS OF EACH VARIETY BASED ON LENGTH OF EMBRYO PROJECTION

Variety	Units of measurement						Average*
	0	1	2	3	4	5	
Canus	20	40	33	7	—	—	1.27
Red Bobs	21	34	36	8	1	—	1.34
Thatcher	5	36	44	15	—	—	1.69
Mindum	8	30	30	24	8	—	1.93
Marquis	5	23	36	29	7	—	2.10
Reward	1	17	46	34	2	—	2.19
Ceres	3	19	35	33	10	—	2.28
Renown	2	9	37	31	17	4	2.64
Apex	—	1	24	53	22	—	2.96
Regent	1	2	18	50	19	10	3.14

* Necessary difference at 5% point is 0.36 and at 1% point —0.48.

The above table shows that the varieties Canus and Red Bobs have short embryo projections, while Regent, Apex and Renown are at the other extreme. with Thatcher, Mindum, Marquis, Reward and Ceres intermediate. There appears to be a definite relationship between the length of projection and the amount of embryo exposure. The records on hundreds of samples were examined in regard to the number showing severe embryo exposure. From these data the Thatcher variety revealed only 1·7% of the samples with severe injury, whereas Regent, Apex and Renown showed 29·2, 33·7 and 34·7% respectively. Marquis showed a value of 20% on a small number of samples. A similar varietal relationship is show in the work of Russell and Ledingham mentioned above, where they included both slight and severe exposures.

Mould Growth and Heating

The importance of mould growth associated with heating in stored damp grain has been reported by many investigators. The significance of mould growth in damp wheat was demonstrated in some work conducted here several years ago (6). It is known that injured grain is readily attacked by moulds under suitable conditions. Bailey (1) has shown that broken or otherwise damaged corn, tends to respire at a higher level than sound grain and that there is an increased risk in storing such grain.

Some tests were made with samples of high and low embryo exposure to determine their susceptibility to mould growth. Almost invariably samples with severe exposure, when placed under conditions of high humidity, soon showed a greater mould growth than the lesser injured samples.

This was particularly noticeable in samples of Regent wheat which had been through a small thresher to increase the exposure. The injured portion showed an embryo exposure, of 98% while the check portion had only 4%, Figure 3. It should be mentioned, however, that in this particular case the injuries were complicated by apparent direct injury to the embryo as shown by weak germination. Furthermore in samples showing

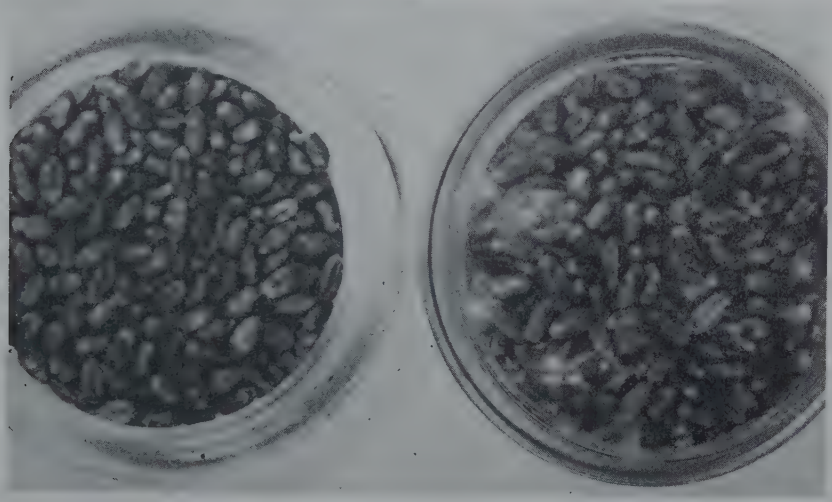

FIGURE 3. Samples of Regent wheat exposed in a moist atmosphere for nine days. The sample on the left which had 4% embryo exposure shows little mould growth while on the right the one with 98% embryo exposure reveals a fair amount.

high embryo exposure, such as the one under discussion, there are always many broken kernels and free starch fragments. The two samples were tested with Fehling's solutiou after soaking for 24 hours. The filtrate from the severely injured sample gave the stronger test, indicating an abundance of reducing sugars or related substances. It is possible that the injuries facilitated the release of enzymes from the kernel tissues. It is expected, that most cases of severe embryo exposure would be attended by complications as mentioned. The invasion by fungi readily follows under favourable conditions.

The extent and progress of heating in damp samples were determined from thermos bottle tests. Many trials were conducted. Two typical experiments with Apex wheat will be outlined in detail. One sample had an exposure of 18% and the other 92% but otherwise they were similar. There was very little direct injury to the embryo in the portion showing high exposure judging by germination results. The germination of the low embryo exposure samples was 97% and for the high exposure 87%. For the first test the wheat was soaked for 25 minutes when moisture determinations were made. The sample with 18% embryo exposure showed a moisture content of 21% and the sample with high exposure showed 23% moisture. The samples were placed in the ice-box for 24 hours while the heat of imbibition was dissipated. Pint thermos bottles in duplicate were then filled with the damp wheat, fitted with a thermometer and plugged

with cotton. For the second test the procedure was much the same, except that after soaking, the wheat was held at room temperature for 24 hours. In this test the sample with low exposure had a moisture content of 24% while the high exposure one had a 25% moisture content. Temperature readings were made daily. The average for the respective duplicates for each test are given in Table 4.

TABLE 4.—TEMPERATURE RECORDS ON DAMP WHEAT WITH LOW AND HIGH EMBRYO EXPOSURE

Days	Temperature in Centigrade			
	Test 1		Test 2	
	Embryo exposure		Embryo exposure	
	18 p.c.	92 p.c.	18 p.c.	92 p.c.
1	19.5	20.0	22.5	23.5
2	20.5	21.5	22.5	24.5
3	21.5	23.0	22.5	25.0
4	21.0	24.5	22.0	26.5
5	21.0	24.0	20.5	26.0
6	21.0	26.5	20.5	27.5
7	20.5	31.5	21.5	30.0
8	20.0	33.5	24.0	34.0
9	20.5	36.5	25.0	38.5
10	20.5	39.0	25.5	41.0
11	21.5	40.5	—	—
12	20.5	41.5	25.5	43.5
13	21.5	44.5	28.0	46.5
14	22.0	45.0	29.5	46.5
15	21.5	45.5	33.0	49.0
16	22.0	47.5	36.5	51.0
17	—	—	38.0	51.0
18	—	—	—	—
19	23.5	51.0	36.5	44.0
20	23.5	50.5	35.5	38.5
21	23.5	49.5	38.5	33.5
22	23.5	46.5	38.5	26.5
23	24.0	42.0		
24	25.0	31.5		

The results of these tests show the rapid increase in temperature in the case of severely damaged samples, Figure 4. An examination of the wheat at the end of the tests revealed an abundant growth of what were considered to be thermophilic fungi along with *Aspergillus* spp. and some *Penicillium*. Examinations at intervals throughout one test showed the appearance of *Penicillium* first, followed by the *Aspergillus* and thermophilic species.

It was concluded that embryo exposure and concomitant troubles might result in serious seed damage under circumstances suitable for fungus growth. This applies particularly to storage conditions. Injury is less likely to occur under similar circumstances with sound seed.

EMBRYO EXPOSURE IN RELATION TO SEED TREATMENTS

From the numerous samples examined several were selected for study in the field. In test 1 Apex wheat was used, the seed was from three different sources, all three, however, being in the same soil zone. The

samples were as nearly uniform as possible with regard to colour, plumpness and germinability. The one outstanding difference between them was their percentage of embryo exposure; this ranged from approximately 15% in the low and 50% in the intermediate to 85% in the high group. In test 2 Regent wheat was selected. As in test 1 three groupings were

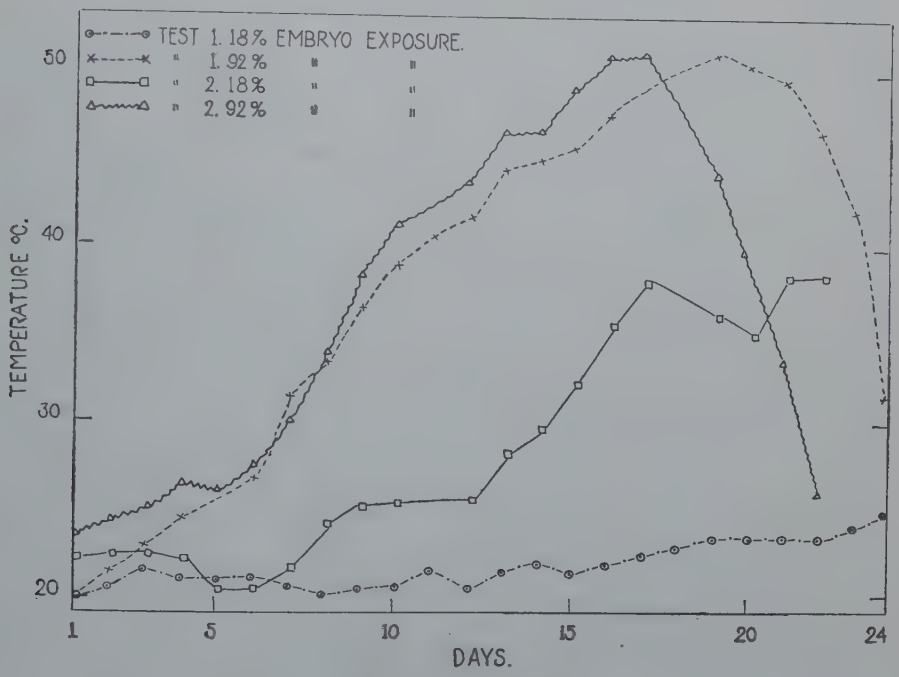

FIGURE 4. Showing the daily changes in temperature as a result of mould growth. Comparing samples with high embryo exposure and many cracked kernels with low embryo exposure and little or no seed breakage.

established as to embryo exposure, viz., low, intermediate and high. The seed in each of these lots, however, was not from a single source. It was a composite sample. Approximately 25 samples, each with a low embryo exposure rating, were taken at random from different points in the province and were mixed together to form the composite low. The same procedure was employed to form the intermediate and high samples. Examination of several hundred seeds from each group after mixing showed the percentages of embryo exposure to be 14, 48 and 70 respectively.

The seed treatments chosen were Lunasan and formalin in test 1 and Ceresan and formalin in test 2. The tests were conducted at both Saskatoon and Indian Head and were carried out in plots each consisting of 5 rows, 18·5 feet in length, replicated 5 times and arranged in the split plot randomized block plan; 250 seeds were sown in each row. Data obtained from the tests were emergence counts and yield of threshed grain. These data are given in Table 5 for test 1 and Table 6 for test 2.

TABLE 5.—THE EFFECT OF EMBRYO EXPOSURE ON THE EMERGENCE AND YIELD OF APEX WHEAT SAMPLES SUBJECTED TO DIFFERENT SEED TREATMENTS

Place	Source of sample	Embryo exposure	Check		Lunasan		Formalin	
			Emergence	Yield	Emergence	Yield	Emergence	Yield
		%		gm.		gm.		gm.
Saskatoon	Elstow	15	216	1174	234	1133	163	1238
	Dodsland	50	217	1207	227	1040	153	1197
	Regina	85	214	1065	228	930	83	870
Indian Head	Elstow	15	226	3146	241	2938	210	3027
	Dodsland	50	227	3379	237	3131	197	3037
	Regina	85	231	3189	241	3309	183	2984

TABLE 6.—THE EFFECT OF EMBRYO EXPOSURE ON THE EMERGENCE AND YIELD OF COMPOSITE SAMPLES OF REGENT WHEAT, SUBJECTED TO DIFFERENT SEED TREATMENTS

Place	Embryo exposure	Check		Ceresan		Formalin	
		Emergence	Yield	Emergence	Yield	Emergence	Yield
	%		gm.		gm.		gm.
Saskatoon	14	205	1050	211	1116	144	1038
	48	199	1109	211	1163	121	1019
	70	199	971	207	945	114	925
Indian Head	14	219	3082	225	3055	197	3188
	48	214	3041	222	3173	175	3075
	70	217	3096	230	3267	175	3115

It will be seen from Tables 5 and 6 that in test 1 the emergence of the Lunasan treated seed and in test 2 the Ceresan treated seed was somewhat higher than that of the untreated seed. Moreover, the degree of embryo exposure did not affect the emergence to any extent when the seed was untreated or treated with either of the organic mercury dusts. Previous work (4) has shown that a progressive decrease in emergence coincident with increasing embryo exposure may be expected. It is not clear why this was not the case in the untreated and mercury dust treated seed in the experiments reported here. It may indicate, however, that the seed samples were particularly free from pathogenic organisms and that soil conditions were favourable for good germination. The formalin treated seed reacted differently. In it there was, generally, a consistent decrease in emergence with each increase in amount of embryo exposure. It is perhaps noteworthy that the emergence of formalin treated seed was higher at Indian Head than at Saskatoon. Further, the differences in emergence, as between the two stations, were most pronounced in seed samples having a high percentage of embryo exposure. It is probable that soil moisture at seeding time was responsible for the difference. Zundel (7) and Hurd (3) have shown that formalin treated seed is more liable to injury when germination is retarded due to lack of moisture. The present report suggests that this is particularly true in the case of seed with a high percentage of embryo exposure. The emergence data was subjected to statistical analysis, the results of which are given in Table 7.

TABLE 7.—ANALYSIS OF VARIANCE OF THE EMERGENCE DATA FOR SASKATOON AND INDIAN HEAD

Source of variance	D.F.	Mean square*	
		Test 1	Test 2
Replications	4	*218*	225
Stations	1	**18547**	**19507**
Treatments	2	**41593**	**35433**
Stations × Treatments	2	**7013**	**4046**
Error I	20	59.7	139.5
Embryo Exposure	2	**2705**	**967**
Embryo Exposure × Treatments	4	**2630**	**536**
Embryo Exposure × Stations	2	**1225**	*124*
Embryo Exposure × Stations × Treatments	4	**665**	62
Error II	48	46	37.7

* Data in bold-face and italics exceed the 1% and 5% levels of significance respectively.

Differences between stations and also differences due to seed treatments were found to be highly significant as judged by the 1% point. With reference to seed treatments, Lunasan in test 1 and Ceresan in test 2 were responsible for increases, and formalin in both tests caused a decrease in emergence, all of which were highly significant. The interaction, stations × treatments was highly significant showing that the variation in environmental conditions as encountered between stations had a decided effect on the emergence under certain fungicidal treatments. Seed treated with Lunasan and Ceresan reacted quite similarly at both stations indicating that the highly significant differences referred to are mainly attributable to the one treatment, formalin.

The response due to different degrees of embryo exposure was highly significant and showed a progressive decrease in emergence with increased embryo exposure. The decrease in emergence with increased embryo exposure can be traced to the formalin treatment, other treatments having only a slight effect. The interaction, embryo exposure × treatments was highly significant. In singling out the effect of the different treatments it was found that there was very little additional benefit from the use of Lunasan or Ceresan on seed with a high percentage of embryo exposure as compared to sound seed. This phase of the results has already been commented on in this report. With formalin on the contrary there is a clear cut reduction in emergence with higher percentages of embryo exposure.

Embryo exposure × stations was highly significant in test 1 and to the 5% point in test 2. Most of this difference is attributable to the formalin treatment and apparently was due to less favourable moisture conditions at seeding time at Saskatoon. Finally, the second order interaction embryo exposure × stations × treatments was significant to the 1% point in test 1 but did not reach the 5% point in test 2. Here again the main factor responsible for these differences was found to be formalin.

From Tables 5 and 6 it may be seen that the yield data presents a different problem in these tests. Differences in emergence were not always reflected in yield. At Saskatoon lowered yields were obtained quite consistently from the severely injured seed regardless of treatment, but

at Indian Head no distinct trend was apparent. Moisture conditions at Saskatoon were poor throughout the growing season while at Indian Head rainfall in general was adequate. It is considered that at Indian Head the fewer plants in some rows were able to utilize the extra space and moisture to such an extent that they produced yields as heavy as rows in which there was only a slight reduction or none at all in emergence. At this time it might be pointed out that the tests at Indian Head were conducted in a field which had been summerfallowed carefully every second year so that the sub-soil moisture supply was good and there were no weeds to compete with the crop. The latter is perhaps an important factor in a test of this nature. It is a condition which does not obtain on the average farm. If there had been weeds present in appreciable numbers there would have been the usual competitive action between weeds and crop, the weeds getting their best foothold in the rows in which emergence had been reduced. Extensive tests by Godel (2) over a wide range of conditions have shown that a light seeding rate in a field relatively free of weeds does not seriously affect the yield of wheat, but where the field is weedy, the yield is lowered considerably. It is considered that the lowered emergence encountered in these tests due to embryo exposure simulates the experiments of Godel where rates of seeding were varied. Therefore, the results of his experimental work would apply to the tests considered in this report.

Figure 5 shows the emergence and yield data for tests 1 and 2 in the form of a bar graph. In Table 8 are given the analyses of variance of the yield data for tests 1 and 2 at Saskatoon. The data for these tests at Indian Head were not analysed along with the Saskatoon yields. Scrutiny of the weights showed no differences either between treatments or percentages of embryo exposure. The yields at Indian Head were almost three times as great as those for Saskatoon which would tend to cover up differences if the two stations were analysed together.

TABLE 8.—ANALYSES OF VARIANCE OF YIELD DATA FROM TESTS 1 AND 2 CONDUCTED AT SASKATOON

Source of variance	D.F.	Mean square*	
		Test 1	Test 2
Replicates	4	**10803**	7546
Treatments	2	1875	983
Error I	8	2060	4404
Embryo Exposure	2	*8730*	**3828**
Embryo Exposure × Treatments	4	1200	269
Error II	24	789	1141

* Data in bold-face and italics exceed the 1% and 5% levels of significance respectively.

From Table 8 it will be seen that the different treatments produced no significant effect on the final yield. Embryo exposure, however, did have an effect, the result being lowered yield with increased percentages of embryo exposure. It proved to be highly significant in test 1 and barely significant to the 5% point in test 2.

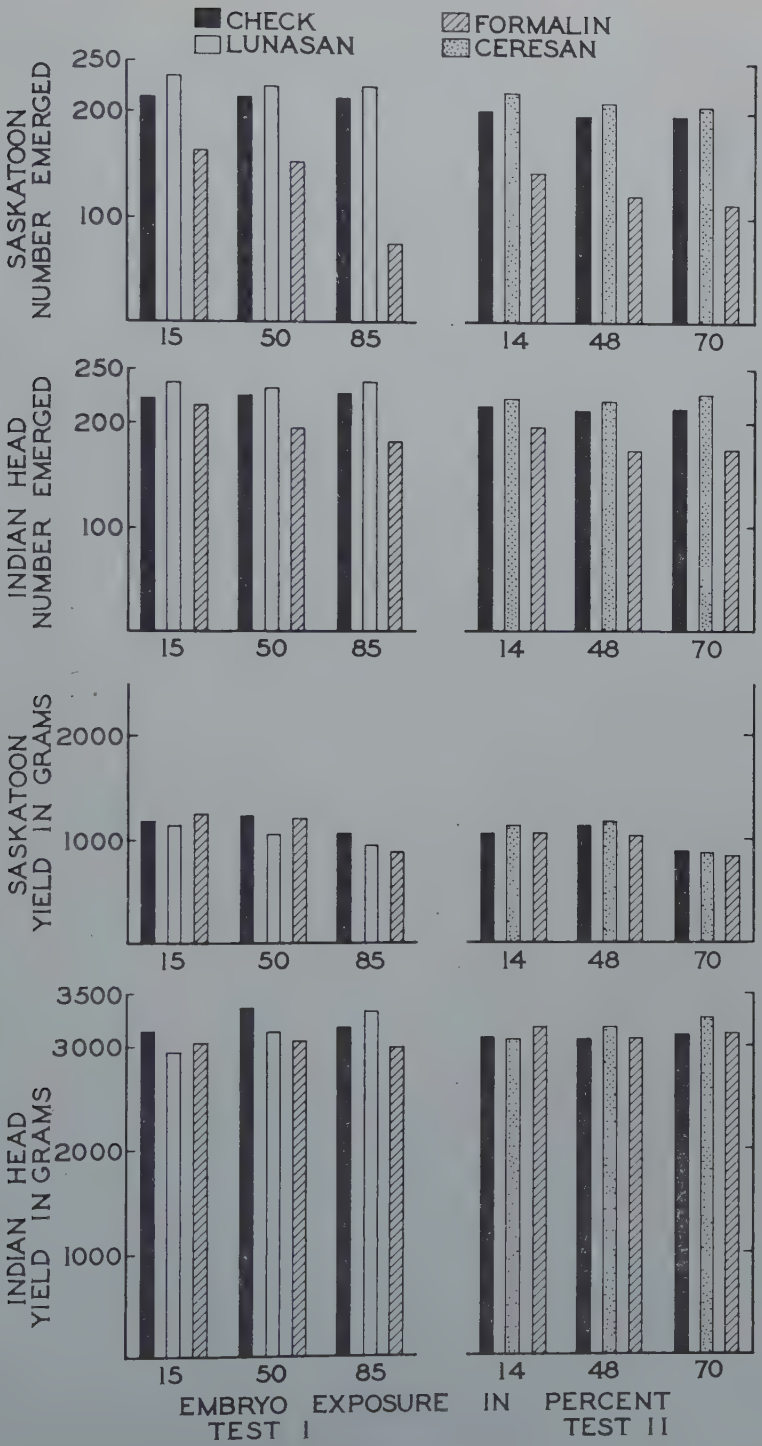

FIGURE 5. Chart showing the emergence and yield data for Tests 1 and 2, at Saskatoon and Indian Head.

Finally, a point of interest was noted when the emergence and yield data were compared in the tests conducted at Saskatoon. From Table 5 it is evident that there was very little difference in emergence between the seed lots having 15% embryo exposure and those with 85%, when they were untreated or treated with a mercury dust, although there was a significant difference in yield. In the untreated seed the effect of severe embryo exposure was a lowering of emergence by only 1% with a subsequent reduction in yield of 9%. In the same test mercury dust treatment further accentuated these differences as shown in a depression of 3% in emergence and 18% in yield. These results are closely paralleled in test 2 conducted at Saskatoon, the data of which may be found in Table 6. Thus, the salient points which the writers desire to stress are: (1) under certain environmental conditions severe embryo exposure is liable to have a more deleterious effect on yield than on emergence, and (2) this situation proved to be further aggravated through the use of mercury seed treatments. These reactions are difficult to explain satisfactorily and need further study. In a similar test at Indian Head where growing conditions were favourable such phenomena were not apparent.

DISCUSSION

A survey of cereal seed samples by means of laboratory tests was again used as a basis for interpreting the seed disease situation in Saskatchewan. An annual survey is valuable for comparisons from season to season. In the present work, however, the samples were all from seed growers and probably were of better grade than the general run. A true annual survey should deal as well with farmers samples properly selected for variety and district. Moreover a general examination of several hundred samples usually brings to light some problems which need special attention and study.

The results of the laboratory tests when compared with the data for 1939 show many similarities. There was a tendency for smudge to increase from soil zone 1 to zone 3. There was less in zone 4 this season (1940). Tests under way at present indicate the advisability of further analysis of the general smudge type of discolouration. The severe form of smudge caused by *H. sativum* appeared, as a rule, in small amounts in recent surveys. Piebald was, as expected, most severe in the northern areas. Embryo exposure was somewhat more prevalent in the samples from the south or drier regions with quite a distinct tendency to be more severe in the varieties Regent, Renown and Apex. As in previous surveys only a small number of wheat samples carried sufficient smut to necessitate treatment for control. On the other hand many of the oat seed samples especially of the more susceptible varieties bore heavy spore loads indicating definitely the necessity of treatment. The barley samples showed moderate amounts of smut, many of which no doubt would benefit from treatment. The importance of seed examinations is clearly seen in regard to the smuts which can be controlled by seed treatments. When a representative sample is examined the results indicate with reasonable assurance the treatment procedure, if any, to adopt. *H, sativum* was found on some of the barley samples. Otherwise the coarse grain samples were relatively free of any disease. It

should be mentioned, however, that barley and oat seeds have not been explored as fully from a disease standpoint as wheat seed.

Embryo exposure in wheat seed, the importance of which was stressed in previous work, was again the subject of further study. The significance of the length of the embryo projection was checked by making measurements and comparing some common varieties. The length of the projection varied from a length of 1·27 units for Canus to 3·14 units for Regent. By comparing the data on severe embryo exposure with length of embryo projection as between varieties a definite relationship appeared to exist. Those varieties with a long embryo projection showed the greatest amount of embryo exposure, with a similar direct relationship between short projections and low exposure injury, in so far as data were available for study.

A few preliminary tests showed that samples with severe embryo exposure soon were covered with a heavy mould growth when exposed to high humidity. Such samples usually contain many broken kernels and starch fragments from the endosperm, all of which would favour fungal development. The significance of the mouldiness was investigated in regard to heating. Several experiments were conducted which clearly revealed a tendency for samples containing high embryo exposure to heat rapidly under conditions of suitable moisture and insulation. The heating was attended by heavy fungus growth in every case.

The samples showing various degrees of embryo exposure employed in the seed treatment tests did not in general reveal great differences. In previous work degrees of injury were about proportional to the extent of embryo exposure. There may be and probably are inherent differences between samples beyond that shown by embryo exposure which must be kept in mind in comparative tests. The experiments, however, did reveal some interesting facts. In the tests run at Indian Head, excellent growing conditions tended to submerge any differences as between degrees of embryo exposure and also between the various treatments. Formalin caused distinct injury especially in regard to emergence. This was not, however, always reflected in the yield for the growing conditions were apparently the limiting factor. The mercury dusts tended to improve emergence and yield in some cases. At Saskatoon where growing conditions were less favourable a slight but distinct difference between the low and high embryo exposure samples was noted. This was mainly reflected in the yield results. In the untreated lots of test 1 there was a difference in reduced emergence of only 1% between the sample showing 15% embryo exposure and the one with 85%. The yield data, however, in comparing the samples in the same manner show a difference of 9%. In the same test the samples treated with Lunasan showed a general increase in emergence but with a difference of reduced emergence of 3% between the samples containing low and high embryo exposure. The yield data for these same two showed a reduction of 18%. A similar relationship was shown in test 2 where Ceresan dust was used. It is difficult to explain adequately the distinct reduction in yield in relation to the emergence in these tests. Apparently growth was retarded in some way following emergence and this was reflected in the depressed yield. This depression reaction was increased by the mercury dusts under conditions of limited soil moisture. These phenomena need further study. In the case of formalin there was an improvement as

the season advanced which can be explained on less competition following a lower emergence. The reduction however was marked where the seed was severely injured.

The two samples compared in these tests were not sufficiently different in respect to injuries to respond significantly to treatment except for one such as formalin. This again emphasizes the importance of evaluating the amount of injury carried by the seed samples before proper seed treatment can be recommended.

SUMMARY

1. A total of 817 samples of wheat, oats, and barley seed grown in Saskatchewan were examined by a series of routine laboratory tests. None of the wheat samples and only a few of the barley and oat samples showed a significant amount of smut as determined by surface washings. Embryo exposure was prevalent especially in certain varieties. Only a small amount of severe smudge was found in the wheat and barley seed. A few minor seed troubles were recorded.

2. Wheat samples with a high percentage of embryo exposure were susceptible to mould growth with subsequent heating under suitable conditions.

3. The wheat samples with low and high amounts of embryo exposure did not show wide differences in field tests except where formalin treatment was used. Differences were more noticeable where soil moisture was limited. The mercury dusts tended to increase emergence and yields in some cases.

4. The Saskatoon tests under conditions of limited soil moisture showed a reduction in yield for the seed having high embryo exposure both for the mercury treated and untreated lots. A similar comparison did not reveal wide differences in emergence.

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STUDIES OF STORAGE BUTTERS SHOWING SURFACE DETERIORATION[1]

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One of the important problems confronting Canadian creamerymen is that of surface deterioration of storage butter. This has been particularly true since Dominion grading regulations (13) were amended to include the examination and grading of the top surface of butter as well as the interior of the box. In most cases, surface deterioration does not extend deeper than one-eighth to one-quarter of an inch, so that only a relatively small proportion of the bulk of a 56-pound box of butter is affected. Reports (8) of grading statistics showed that, during the years immediately following this change, there was a considerable increase in the percentage of storage butter which lost grade on regrading, and it was estimated that surface defects were responsible for approximately 50% of this butter being lowered in grade after storage. This is of considerable economic importance to Canadian creamery operators because much of the butter which was lowered in grade after storage would have maintained the original grade had it not been for surface deterioration.

TYPES OF SURFACE DEFECTS

A common and troublesome surface defect on storage butter is that of a "woody" flavour imparted to the butter by the wood of the container. Investigations (2, 26, 27, 28, 29, 48, 58) concerning the effect of type of lumber, the degree of seasoning, temperature of storage, and packaging materials have indicated that this flavour can be largely controlled by using boxes made from sound, well-seasoned, non-odorous lumber such as spruce, by treating the inside of the boxes to obtain an impermeable coating as afforded by the casein-formalin treatment, by the use of double parchments of suitable weight or an aluminum foil parchment wrapper, and by placing the butter in low storage immediately after manufacture. (Aluminum foil parchment liners are not available at present due to war-time restrictions.)

During storage, butter also frequently develops other surface defects variously described as unclean, stale, tallowy, metallic, oxidized, fishy, etc., which are usually of a complex nature and may be due to a number of factors. From the extensive literature on the subject, the chief factors affecting butter quality and deterioration may be summarized briefly as follows:

1. The use of poor quality cream, due to feed and weed flavours, and those caused by microbiological activity.
2. The action of micro-organisms in the butter itself.
3. Enzymatic activity in the butter.
4. Chemical changes in the butter constituents, especially the fats or related materials, such as lecithin.

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It is logical to assume that butter deterioration caused by the last three of the above factors would be intensified and hastened at the surface of the butter where conditions are more favourable for the growth of bacteria, and for enzymatic activity and chemical changes.

The importance of high quality cream in relation to the initial and keeping quality of butter is now generally recognized. Butter made from sour cream in which there has been considerable microbial activity does not have as good keeping quality during storage as butter made from high quality raw materials. This fact is clearly indicated in Canadian grading statistics (8) which show that a considerably smaller percentage of the butter with an original flavour score of 40 lost grade after storage than butter of original 39-score for flavour. No discussion, therefore, of flavour defects and the deterioration of butter during storage can ignore the fundamental question of cream quality.

In work reported on the bacteriology of butter held under modern commercial storage conditions, there does not appear to be any close correlation between the plate count of micro-organisms and the keeping quality of butter during storage. Hammer (21) states that "at commercial storage temperatures of 0° F. or lower, organisms do not develop in butter and the defects that appear are due primarily to chemical action". Loftus Hills *et al.* (37), Arup and Gilmour (1), Sadler and Vollum (53), Grimes (19) and Nelson (44) could find no definite correlation between the total bacterial count and the flavour score or grade of storage butter. Jacobsen (33) reported that after storage at −25° C. (−13° F.) for 90 days no lipolytic or proteolytic bacteria were detected in salted butters even after holding for 7 days at 21° C. (70° F.) and the predominant flavour defect was that of tallowiness. No correlation appeared to exist between bacteriological content and keeping quality. Naylor and Guthrie (43) also considered that bacteria were of little significance in the deterioration of commercial salted butter as indicated by the total bacterial counts or counts of caseolytic organisms. On the other hand, when butter is held at temperatures which permit the growth of bacteria, certain types of bacteria have been found to be related to butter defects. Guthrie, Scheib and Stark (20) state that "in the absence of other spoilage factors, a direct correlation seems to exist between the numbers of fat-splitting and casein-digesting bacteria present and the spoilage of butter". Hammer and his associates (11, 12, 16, 22, 23, 32) in their investigations on the bacteriology of butter have shown that butter defects may be caused by specific types of bacteria. Ruehle (52) also believed that many organisms were capable of producing off-flavours in butter. Sorensen (55) in keeping quality tests of commercial butters found that putrid and cheesy flavours were most frequently encountered and were traced to contamination of the butter with spoilage types of bacteria from creamery water supplies. Shutt (54) considered that putrefactive organisms in water supplies were responsible for the surface flavours of butter. Wolochow, Thornton and Hood (61) have shown the importance of *Pseudomonas putrefaciens* to the development of "surface taint". Hood and White (30) in earlier studies showed that, in general, butters showing "surface taint" contained large numbers of bacteria, many of which were able to liquefy gelatin. Where bacterial counts have been

made of the surface and interior of the same butters, it has been found that in nearly every instance the counts are considerably higher on the surface (16).

Investigations of the action of micro-organisms on fats by Jensen and Grettie (34) and Castell and Garrard (9) indicate that organisms producing oxidase and lipolytic enzymes may bring on rancid or oxidative flavours, and Castell (10) has shown that butter showing cheesy and "surface taint" flavours contained high numbers of oxidase-positive organisms. He found most of the oxidase-positive bacteria were gram-negative rods of the groups *Pseudomonas* and *Achromobacter* and considered them to be important in butter spoilage as they are known to produce some of the common butter defects and are able to grow at comparatively low temperatures.

Defects of enzymatic origin are uncommon in butters with present day methods of manufacture. The high temperature of pasteurization (at least 170° F. for 10 minutes) used in Canadian creameries destroys the enzymes present in the cream, including lipase, which is responsible for rancid and bitter flavours. The possibility of butter defects resulting from products of enzymatic action in the cream before pasteurization should not be overlooked, and there is some evidence (9, 10, 34) that oxidizing enzymes of bacterial origin might be a factor in butter deterioration. Wiley (59), from the results of studies on the oxidation in fat of butter during cold storage, suggests that there is an oxidizing enzyme present in ripened pasteurized cream and in unripened raw cream which is most active at low pH values and high salt concentrations.

Flavour defects in butter, described as stale, storage, oxidized, tallowy, lardy, oily, fishy, etc., are now generally associated with chemical changes of an oxidative nature in the fat or related constituents such as lecithin. From comprehensive reviews of the literature on this subject by Lea (36) and Brown and Thurston (7), the chief factors responsible for this type of chemical change in fat are:

1. The composition of the fats; the higher the percentage of unsaturated tri-glycerides, the greater is the susceptibility of the fat to oxidation.
2. Excessive acidity in the butter which may act as a pro-oxidant.
3. Contamination with traces of heavy metals. Copper is the most important metal in catalyzing the oxidative reaction.
4. The catalytic effect of light. Exposure to sunlight often brings on "tallowy" or "oily" flavours.
5. Temperature of storage. The higher the temperature the faster will be the rate of oxidation.
6. The amount of surface exposed to atmospheric oxygen.

All of these factors are closely related in initiating and accelerating oxidative changes in the fats, and the flavour defects resulting from such reactions may be due to a combination of two or more of the above factors. Most of these factors, however, can be controlled to some extent by the creamery operator during the processing and manufacturing procedures, by proper packaging. and by suitable storage conditions so that deterioration of the butter surfaces may be greatly lessened.

THE OBJECT OF THE STUDY

In view of the many factors which may be involved in the deterioration of the surface of first grade butter during storage, it was thought that a bacteriological and chemical study of some storage butters which had been lowered in grade, solely because of surface defects, might yield information that would be of value in indicating some of the main sources of trouble.

PROCEDURE AND METHODS

Collection of Samples

Butters having surface defects, other than woody flavours, intense enough to cause a loss of grade even though the interior was still first grade quality, were sampled from the 56-pound box by members of the Dominion grading staff at the time of regrading. A portion of the top surface, approximately 5 inches square and 1½ inches thick, was cut away from one corner of the box. This piece was cut in halves and the inner surfaces placed together. The butter was wrapped in clean parchment, packed in a shipping carton and refrigerated at low temperatures before shipment to prevent undue growth of organisms at the surface. The samples were sent by express to the laboratory and were usually received in two or three days from the time of shipment. Every precaution was taken by the graders to avoid contamination during sampling and the samples were plated immediately on arrival or stored at temperatures below 32° F. until analyzed.

At the laboratory thin layers of the surface butter were scraped off with sterile metal spatulas or wooden tongue depressors into sterile glass bottles for both bacteriological and chemical analysis. Samples of the unaffected interior portion of the butter were taken after the surface portions were removed.

A few samples of butter which had maintained the original grade during storage were also collected and sent in the same way. Information regarding the original grade, description of the defect, the time and temperature of storage, and date of manufacture accompanied each sample.

Bacteriological Analysis

Bacteria counts were made by the plate method using beef infusion agar (pH 6.8) for the first 14 samples and tryptone glucose extract agar (56) for the remainder. Sterile skimmilk and sterile fat emulsion (38) were added to the melted agar at the rate of 5%, or approximately 0.5 cc. of each were added to each petri dish just before pouring. In addition to total counts, this method enabled the numbers of proteolytic and lipolytic organisms to be counted. Duplicate plates were poured for each dilution and incubated for 5 days at room temperature (70° to 77° F.). The number of oxidase-positive colonies was determined by flooding one of the countable plates with a 0.5% solution of *p*-aminodimethylaniline monohydrochloride as suggested by Castell and Garrard (9).

The general types of organisms present on the surface and interior portions of the butter were determined by picking 25 contiguous colonies from a plate into tubes of sterile litmus milk and incubating for 14 days

at room temperature. The various changes that occurred were described as acid coagulating, acid non-coagulating, proteolyzed, alkaline, or inert. Microscopic examinations of gram-stained smears of colonies from the plates and of Breed smears of the curd were made to indicate the predominant morphologic types present in the surface and interior samples.

Chemical Analysis

pH determinations were made on the serum of the butter with the quinhydrone electrode for the first 14 samples and with the glass electrode (Beckman pH meter) for the remainder. The serum was collected by centrifuging melted butter and drawing off the fat. Peroxide values of the fat were determined by a modification of Lea's method (36) as used by Holm *et al.* (25). Analyses for copper content were made by the Division of Chemistry, Science Service, using the filtration method of Moir and Andrews (41).

DATA ON THE SAMPLES STUDIED

The information obtained on the butter samples studied is given in Table 1. All samples were first grade when originally graded, with 17 having a 40-score for flavour. Altogether 45 samples were analyzed, 38 of which had been lowered in grade for surface defects although the bulk of the butter was still first grade after storage, while 7 had maintained the original first grade quality. Of the 38 samples degraded, 12, or 31.5%, were originally 40-score butter, while 5, or about 71%, of the 7 samples which showed no loss of grade were 40-score butter. The samples represented commercial butters made in Alberta, Saskatchewan, Manitoba, Ontario, and Quebec, and the majority of the churnings were made during June, July, and August, with a few made in April, May, September, and October. From a number of determinations, the moisture content of the butter ranged from 14.2 to 15.9%, and the salt content ranged from 1.03 to 2.0%.

The flavour defects present were described as unclean, stale, storage, oily, tallowy, lardy, oxidized and fishy, which are flavours usually associated with changes in the fat of an oxidative nature. Three of the samples developed surface defects of sufficient intensity to lower the grade about one week after manufacture, but the remainder had been in storage for periods of from 32 to 289 days. The butters which maintained their original quality had been stored for periods of $2\frac{1}{2}$ to over 16 months.

Storage temperatures ranged from 15° F. to −20° F. with the majority of butters held at temperatures between 5° F. and −5° F. Two of the samples which deteriorated on the surface in about one week were held at temperatures of 33° F. and 50° F.

Most of the butter had been packed in the usual paraffined boxes and wrapped in double 40-lb. parchment liners, with an extra headpiece of parchment placed on the top surface of the butter in a number of cases. At least one sample had been packed in a casein-formalin treated box and three samples had been wrapped in aluminum foil parchments.

TABLE 1.—DATA ON BUTTER SAMPLES STUDIED

Lab. No.	Flavour scores		Month of mfg.	Days in storage	Temp. of storage	Graders' remarks on surface defects
	Original	Regrade				
Samples degraded for surface defects						
					°F.	
1	39.0	38.0	July	120	— 5	Unclean, oily
2	39.0	38.0	Aug.	108	4	Unclean, oily, tallowy, sl. fishy
3	39.0	38.0	Sept.	46	15	Sl. tallowy
4	39.0	—	Sept.	32	5	Sl. tallowy
5	39.0	38.0	July	113	— 5	Very unclean, fishy tendency
6	39.0	38.0	Oct.	8	10	Stale, unclean
7	39.0	38.0	July	122	10	Unclean, sl. metallic, fishy tendency
8	40.0	37.0	July	125	— 2	Tallowy
9	39.0	38.0	July	131	10	Unclean, tallowy, fishy tendency
10	39.0	38.0	June	132	10	Oily, stale
11	40.0	38.0	June	153	2	Tallowy, stale
12	40.0	38.0	Aug.	91	5	Objectionable, sl. tallowy
13	40.0	38.0	Sept.	92	— 3	Oxidized or tallowy
14	40.0	38.0	Sept.	90	— 3	Oxidized or tallowy
16	39.0	38.0	June	155	—12	Sl. stale
17	39.0	37.0	June	154	—12	Stale
18	39.0	38.0	July	116	2	Oily, tallowy
19	39.0	38.0	May	183	2	Oily, tallowy
20	39.0	38.0	Aug.	104	—10	Tallowy, objectionable
22	39.0	35.0	July	140	— 5	Fishy
23	40.0	36.0	June	149	— 5	Sl. fishy, unclean
24	40.0	38.0	June	198	— 5	Sl. fishy
25	40.0	38.0	June	194	— 5	Tallowy
27	—	36.0	Oct.	64	—	Lardy, very tallowy
28	40.0	38.0	June	198	0	Lardy, objectionable
29	40.0	38.0	Aug.	188	10	Sl. stale
30	—	38.0	Jan.	8	33	Unclean
31	—	38.0	June	215	— 8	Unclean—sour vinegar surface
32	39.0	38.0	July	222	5	Skunk grass, tallowy
35	39.0	38.0	Aug.	289	0	Sl. stale, sl. sour
37	39.0	38.0	July	254	10	Oily, sl. unclean
39	39.0	37.0	July	251	10	Unclean
40	39.0	38.0	June	275	10	Tallowy
41	39.0	37.0	June	275	10	Tallowy, unclean
42	39.0	38.0	July	254	10	Unclean, storage
43	40.0	38.0	July	257	4	Oily, sl. unclean
44	40.0	38.0	Oct.	170	4	Sl. stale
45	—	38.0	April	6	50	Unclean
Samples which maintained first grade quality						
15	40.0	40.0	June	509	0	Very good
21	40.0	40.0	Sept.	83	—20	Fine quality
26	40.0	39.0	July	160	— 5	Very clean
33	40.0	40.0	Aug.	200	3	Fine quality
34	39.0	39.0	Aug.	191	5	Very sl. stale on surface
36	39.0	39.0	July	270	5	—
38	40.0	39.0	July	227	7	—

RESULTS OF BACTERIOLOGICAL ANALYSES

Total plate counts and counts of proteolytic, lipolytic and oxidase-positive bacteria are given in Table 2. Of 36 samples of butter degraded for surface defects, 29 had higher total counts on the surface than the interior, and for 16 of these samples large differences were noted. For

TABLE 2.—PLATE COUNTS OF BUTTER SHOWING SURFACE DETERIORATION

Lab. No.	Total count*		Proteolytics		Lipolytics		Oxidase-positive	
	Interior	Surface	Interior	Surface	Interior	Surface	Interior	Surface
1	1 265,000	1,350,000	50,000	65,000	22,500	16,000	58,000	127,000
2	6,150	3,100	350	100	0	0	100	100
3	8,100	156,000	150	650	900	—	0	12,500
4	19,150	1,050,000	150	6,500	0	11,500	1,900	6,700
5	3,750	213,000	100	1,500	0	2,000	400	27,000
6	41,150	66,500	450	8,500	800	800	6,300	17,400
7	52,500	92 500	5,500	4,500	650	250	1,600	700
8	20 700	63,000	250	550	250	750	2,800	15,600
9	120,500	310,500	13,500	10,500	50	12,500	1,800	14,400
10	2,150 000	2,880,000	165,000	130,000	8,000	24,500	288,000	195,000
11	14,850	104,500	—	—	250	3,000	2,000	—
12	22,200	625,000	9,000	85,000	0	0	0	1,000
14	61,500	390,000	0	20,000	0	25,000	—	—
16	9,000	8,600	2,200	1,800	0	0	0	0
17	229,000	208,500	23,000	34,000	0	0	0	0
18	14,900	104,000	2,150	17,000	0	500	0	0
19	17,500	75,500	0	0	0	0	100	1,000
20	124,000	201,000	13,000	26,000	0	0	14,000	—
22	334,000	368,000	3,000	3,000	0	0	0	1,000
23	20,500	16 500	2,000	1,500	2,500	2,000	2,100	4,100
24	8,900	4,300	0	0	0	0	500	6,000
25	2,700	3,700	150	100	0	0	300	400
27	12,500	34,500	550	1,150	0	1,100	8,200	24,900
28	12,100	114,000	1,150	14,000	0	0	0	15,500
29	189,000	181,000	3,500	3,500	2,500	1,000	3,000	10,000
30	134,500	845,000	12,500	100,000	2,500	85,000	24,000	490,000
31	1,625,000	1,460,000	125,000	120,000	0	0	120,000	230,000
32	41,000	52,000	3,000	550	0	0	0	700
35	32,000	36,000	400	3,500	0	0	3,700	2,100
37	24,000	68,500	1,700	10,500	200	0	2,700	4,000
39	6,500	7,800	650	200	350	300	3,600	4,300
40	14,000	45,000	300	6,500	6,700	22,000	10,500	23,000
41	3,500	17,200	500	2,000	100	1,200	400	1,100
42	26,000	33,000	7,000	12,000	100	200	700	600
43	19,000	26,000	350	1,300	0	200	0	300
44	15,000	326,000	200	4,500	0	12,000	0	10,000
45	88,000	—	0	—	350	—	4,500	—
Samples which maintained first grade quality								
15	26,500	21,000	2,250	1,800	0	0	3,500	4,500
21	9,000	11,000	800	700	0	0	—	—
26	23,500	—	500	—	4,600	—	6,400	—
33	8,000	11,000	700	400	100	450	3,500	5,000
34	30,000	31,000	350	550	8,200	2,300	13,500	13,500
36	48,000	49,000	5,000	2,500	3,500	5,700	18,000	20,000
38	148,000	116,000	4,500	6,000	—	—	32,500	36,000

* Bacterial per ml.

the remaining 7 degraded samples, the total counts of the surface portion were slightly lower than those from the interior. Total counts of the surface and interior butter of the samples maintaining the original grade were practically the same, and in general were quite low. In a number of the degraded samples the total counts were greatly in excess of the numbers which might be expected in butter made from well-pasteurized cream under proper sanitary conditions. The relatively high counts found on samples 1, 10 and 31, and on the surface of some of the other samples, as compared to the interior would indicate gross contamination during manufacture and packing, or considerable growth of the organisms present at some time between packing and regrading. As all samples were sent to the laboratory without refrigeration during transit, temperature conditions may have been satisfactory for bacterial growth, but the method of shipment could hardly account for the high numbers of organisms found in some samples when others reached the laboratory with low counts both on the surface and interior portions.

In some butters, especially those with high total counts, relatively high numbers of proteolytic, lipolytic and oxidase-positive organisms were present on both the surface and interior portions of the samples. In a number of cases, the counts of these types of bacteria were higher on the surface butters, and in a few samples were found on the surface when they were absent from the interior portion. In general, however, the numbers of these types of organisms were so low even on the surface portions of the butter that it is considered unlikely that they were a significant factor in the deterioration of the butter during storage. The relation of the oxidase-positive group of organisms to flavour defects of an oxidative nature is being studied further.

From the results obtained on the relatively few samples studied, there did not appear to be any close correlation between the relative number of organisms on the surface and in the interior of the butters, and the type of defect after storage. For example, in samples 30 and 31, where the counts were high the defect was described as unclean, but the same defect was found on samples 39 and 41 where the counts were relatively low. Flavour defects described as tallowy, oily and stale were found on the surface of butters with both high and low counts. The same relation holds true for counts of proteolytic, lipolytic and oxidase-positive organisms and the particular surface defects of the butters studied. Nevertheless, it is probable that high numbers and the presence of certain types of organisms on the surface of some of the butters may have been a contributing factor to the flavour defects that were present at the time of regrading.

This is indicated by results obtained on keeping quality tests made on a number of the samples. These tests were made by taking a portion of the interior of the butter and holding in a sterile glass bottle at 70° F. for 7 days. Such tests (33, 43, 46) are used rather extensively with considerable success to indicate the probable keeping quality of the butter during storage or while it is in retail trade channels. Of 31 samples tested, 13 remained satisfactory in flavour quality while the remainder developed tallowy, unclean and, in a few cases, cheesy flavours. In the majority of cases, where the flavour remained satisfactory, the total plate counts as

well as counts of proteolytic, lipolytic, and oxidase-positive organisms were relatively low on the interior portions of the butter samples. In samples 1, 9, 10, 30 and 31 which developed unclean flavours, both total counts and counts of proteolytic, lipolytic and oxidase-positve organisms were comparatively high. (See Table 2.) Some samples developed tallowy flavours even though the bacteria counts were low, but in other cases the counts were high, especially at the surface. These results indicate that when butter is held at a suitable temperature, the growth of some types of bacteria may result in undesirable flavours.

TABLE 3.—GENERAL TYPES OF ORGANISMS IN SURFACE DETERIORATED BUTTERS

Sample No.	Percentages of organisms from interior					Percentages of organisms from surface				
	Acid		Proteo-lyzers	Alkali formers	Inert	Acid		Proteo-lyzers	Alkali formers	Inert
	Coag	Non-coag.				Coag.	Non-coag.			
1	14	3	16	—	67	—	92	8	—	—
2	6	53	10	—	31	—	67	3	—	30
3	7	13	3	—	77	6	78	—	16	—
4	3	74	9	6	8	3	43	23	3	28
5	6	76	—	3	15	3	20	18	—	59
6	—	57	3	—	40	—	9	51	—	40
7	—	25	16	—	59	9	78	9	4	—
8	—	17	—	—	83	—	19	7	—	74
9	12	44	9	12	23	6	54	3	15	22
10	—	53	7	3	37	3	45	3	4	45
16	19	69	4	8	—	17	64	4	4	11
17	24	40	4	8	24	24	24	8	16	28
18	—	44	—	16	40	—	48	—	21	31
19	4	50	—	15	31	4	60	—	—	36
20	—	40	4	4	52	4	60	—	32	4
22	—	88	—	8	4	4	84	—	4	8
23	8	4	4	—	84	—	12	8	4	76
24	—	63	—	33	4	—	68	16	12	4
25	—	64	4	8	24	—	64	8	4	24
27	8	64	4	4	20	—	40	12	20	28
28	4	20	4	—	72	—	8	8	48	36
29	4	72	—	—	24	4	50	—	—	46
30	4	16	12	56	12	—	4	4	44	48
31	—	28	—	12	60	—	31	4	19	46
32	4	81	11	4	—	—	88	4	8	—
35	4	—	—	48	48	4	—	4	44	48
37	4	52	—	16	28	12	32	—	40	16
39	4	42	13	16	25	8	44	—	40	8
40	4	21	—	—	75	—	54	4	—	42
41	—	88	—	—	12	31	50	4	11	4
42	12	72	—	—	16	—	76	—	4	20
43	8	68	8	—	16	8	60	4	4	24
44	16	48	—	4	32	—	32	—	4	64
In Butters which had maintained quality										
15	—	40	8	24	28	—	28	—	24	48
26	12	19	—	19	50	—	—	—	—	—
33	4	35	—	—	61	—	16	4	12	68
34	—	4	—	—	96	—	100	—	—	—
36	8	16	4	24	48	16	8	4	20	52
38	—	21	—	33	46	4	33	—	21	42

THE GENERAL TYPES OF ORGANISMS PRESENT

The general types of organisms present as determined by the litmus milk tube method are shown in Table 3. In the samples studied, the largest percentages of the organisms were acid non-coagulating or inert forms, although in a few samples considerable percentages of proteolyzers or alkali formers were present. In many cases the acid or alkali reactions were so slight that there was not much noticeable change from the controls. In general, there were few cases where there were conspicuous differences in the types of organisms found on the surface and interior portions of the butter as shown by their reactions in milk. In some cases the proteolytic and alkali forming types were more abundant on the surface portions while in other cases the reverse was true. There appeared to be no correlation between the percentage of these types of organisms and surface deterioration of the butter. The percentages of the various types of organisms found in butters which had been degraded for surface defects were much the same as found on butters which had maintained their original grade. In the majority of cases the odours produced in milk by the various organisms were not particularly noticeable, but some of the proteolytic types developed objectionable odours of a putrefactive nature. Of interest was the isolation of a gram-negative proteolytic rod from the surface of one sample which produced an odour in milk similar to that produced by *Ps. putrefaciens*, one of the organisms responsible for the specific defect "surface taint" (11, 16, 61).

The most common morphologic types found in the samples, as determined by microscopic examination of smears of the butter serum and of contiguous colonies from the plates, were gram-positive coccus forms. Gram-positive and gram-negative rods were also regularly found but except in a few cases were not the predomlnant types. There were no noticeable differences in the types found on the surface and interior portions for samples which were degraded and those which held their grade during storage. In a few instances spore formers were present, but were not numerous.

RESULTS OF CHEMICAL STUDIES

Data relating to pH, copper content and peroxide values of the butters analysed are given in Table 4.

Acidity has long been known to be an important factor in the deterioration of butter flavours, and investigations (25, 42, 45, 47, 49, 50, 57) have shown that as the acidity of the cream, and therefore of the butter, increased the keeping quality of the butter decreased. In studies relating to the pH of the serum of butter and keeping quality there is further evidence that acidity is closely connected with deterioration of a chemical nature. Arup and Gilmour (1, 18) and Gilmour (17) have shown that there was a decided tendency for the flavour score of butter to decrease as the pH decreased, i.e., acidity increased. These workers recommended that a pH of not less than 6.7 should be maintained in butter for storage. McDowall *et al.* (39) from a study of 202 experimental and commercial butters found that the range in pH was from 6.0 to 8.5. There was no indication of loss of

TABLE 4. —CHEMICAL STUDIES OF BUTTERS SHOWING SURFACE DETERIORATION

Sample No.	pH	Copper	Peroxide values No. cc. 0.002 N. sodium thiosulphate per cc. of fat	
			Interior	Surface
		p.p.m.		
1	6.42	—	—	—
2	6.03	—	—	—
3	6.24	—	—	—
4	6.67	—	—	—
5	6.28	—	—	—
6	7.15	—	—	—
7	5.91	—	—	—
8	6.44	—	—	—
9	5.56	—	—	—
10	6.75	—	—	—
11	6.43	—	—	—
12	6.44	—	—	—
13	7.54	—	—	—
14	6.30	—	—	—
16	6.50	0.14	0.0	0.0
17	6.20	0.17	0.0	0.4
18	6.98	0.13	0.0	0.6
19	5.88	0.10	0.4	1.0
20	6.40	0.14	0.3	0.4
22	5.98	0.80	0.9	0.9
23	5.78	0.28	0.6	0.8
24	5.76	0.09	0.4	0.7
25	6.14	0.10	0.2	0.6
27	6.74	0.12	0.0	0.0
28	6.35	0.10	0.0	0.5
29	5.82	0.11	0.5	1.0
30	6.25	0.10	0.0	0.8
31	6.24	0.07	0.0	1.0
32	7.20	0.18	0.8	1.3
35	6.03	0.09	1.0	1.5
37	7.56	0.09	0.9	1.0
39	6.23	0.12	0.7	0.7
40	6.40	0.12	0.6	0.7
41	6.42	0.09	0.0	0.0
42	6.98	0.07	0.0	0.5
43	6.05	0.10	0.0	0.2
44	5.95	0.10	0.0	0.3
45	5.65	0.09	0.0	0.0
		Samples maintaining grade		
15	6.41	0.10	0.55	0.7
21	7.10	0.16	0.0	0.0
26	6.67	0.12	0.0	0.6
33	7.20	0.11	1.0	1.0
34	7.15	0.09	0.9	0.7
36	7.66	0.12	0.8	1.1
38	5.94	0.12	0.9	1.0

flavour at pH 6.8 to 7.0 and they recommended a pH of 6.7 to 7.2 for storage butters. These workers found that oiliness and fishiness may develop when the acidity of the cream was higher than 0.3%. Loftus Hills *et al.* (37) concluded from their studies that "in order to obtain the

best keeping quality in storage, the butter should be at a pH of about 7.0". Bird, Fabricius, and Breazeale (4) found that when the initial pH of butter ranged between 6.7 and 7.5 there was less development of flavour defects and better maintenance of score. Bird (5) in a later report states that "the effects of acidity of the serum of the butter made from sour cream show rather definite relationships to butter quality". He found that a pH of 6.7 to 7.4 gave the least objectionable flavours while at pH ranges below 6.6 metallic flavours became more important and that fishy flavours are important defects at pH 6.0 or lower. Kretchmar (35) noted that pH was not only correlated with initial flavour scores but also with keeping quality. Butters having the higher initial flavour scores had the higher pH values while with 236 churnings of commercial butters held at temperatures of 10° F. for 8 to 11 weeks, those with a pH range of 6.7 to 6.9 lost less in score than those of lower pH. This worker concluded that the optimum acidity for keeping quality is about pH 6.7 to 6.9.

In these studies the pH values of the butters which showed surface deterioration ranged from 5.56 to 7.56, while those of the 7 butters which maintained quality were between 5.94 and 7.66. While surface flavours developed over a wide pH range, it should be noted that 28 of the 38 butters degraded had pH values less than pH 6.7 and that 12 had pH values of 6.05 or lower. With the 7 samples which did not show surface defects, 5 had pH values of 6.67 or higher and 4 of these were over 7.0. An interesting feature of these data is that samples 2, 5, 7, 9, 22, 23, and 24, which had a fishy flavour or showed a tendency towards fishiness, had relatively low pH values ranging from 6.28 to 5.56 with 5 of the 7 samples having pH values of less than 6.0. The fishy flavour was particularly noticeable on samples 22 and 23 and had permeated the butter. These samples not only had high acidities as indicated by pH values of 5.98 and 5.78, but the copper content was also relatively higher than for the other samples. The combined effect of low pH and the relatively high copper content was no doubt responsible for the development of fishy flavours in these two butters.

The importance of traces of heavy metals, especially copper and iron, in promoting oily, oxidized, tallowy and fishy flavours in salted butter has been realized for many years (31, 51). It is now generally recognized that these metals act as catalysts in the oxidation of the fat or lecithin which is responsible for some of the more important off-flavours in storage butters. The minimum amount of copper necessary to catalyze the oxidative changes in butter fat may vary under different conditions but a number of investigators have shown that minute quantities are sometimes sufficient. Williams (60) found the copper content of both "superfine" and second grade New Zealand butter to be 0.2 to 0.25 parts per million. Davies (15) showed the copper content of New Zealand and Australian butters which had developed tallowy and fishy flavours to vary from a trace to 3.6 p.p.m. However, other factors evidently entered into the development of the defects as butters showing no taint had a higher copper content (1.2 to 1.8 p.p.m.) than some fishy samples. This author considers that high acidity in combination with metallic contamination greatly accelerates the onset of such defects. Moir and Andrews (41) in a study of 373 New Zealand butters found the copper content to range from less than 0.1 to over 1.0 p.p.m. with over 64% of the samples containing less than 0.2

p.p.m. They suggest that if the copper content of butter could be kept below 0.15 p.p.m. and the iron content below 1.5 p.p.m. there would be little trouble from oxidation due to metals. Briggs (6) showed that 0.1 p.p.m. of copper greatly shortened the induction period in the oxidation of butterfat, and that even as low as 0.025 p.p.m. of copper had an appreciable effect in hastening the change. Barnicoat and Palmer (3) found that 0.1 p.p.m. of copper regularly brought on oxidation in butterfat and state that amounts as low as 0.1 p.p.m. of ferrous iron and 0.01 p.p.m. of copper are effective as oxidative catalysts. Harvey (24) considered that butters containing less than 0.3 p.p.m. of copper would not undergo deterioration under normal marketing conditions.

The copper content of the butters examined in the present study ranged from 0.07 to 0.8 p.p.m. In only two samples, both of which had developed definite fishy flavours, was the copper content higher than 0.2 p.p.m. and in about 84 per cent of the samples the copper content was less than 0.15 p.p.m., the limit suggested for storage butters in New Zealand. Nevertheless, the amount of copper was considerably higher than the minimum amounts necessary to induce oxidative changes under some conditions. There was no noticeable difference in the copper contents of the butters degraded for surface defects and those which had maintained the original grade after storage. However, even the small amounts of copper present in the samples showing surface deterioration when combined with the relatively high acidities of some of the butters may have been an important contributory factor.

Among the many chemical tests devised to determine the oxidative changes in fats, the peroxide number is considered (14, 36) one of the more sensitive and has been used by some investigators in the study of butter deterioration. Holm *et al.* (25) state that "there is a direct relationship between the rate of oxidation and loss in score, or stated more directly, the oxidation reaction seems to underlie the various changes that are responsible for loss of score". Lea (36) proposed modifications of the test for routine work and has used it extensively in studying the factors responsible for the deterioration of edible fats including butterfat, but states that "the ultimate standards to which all data must be referred are based on taste and smell". It is probable that in some cases a defect may be apparent to the senses of taste and smell before the changes responsible for the defect can be measured by any of the known chemical tests.

In these studies, the peroxide test was applied to surface and interior portions of the butter samples to determine quantitatively the extent of the oxidation which had taken place during storage. In the majority of cases the peroxide values for the surface were higher than for the interior portions. In some samples the interior portion gave no peroxide reaction while the surface butter gave very definite values. In a few cases no peroxide reaction was obtained even on the surface portions. The average peroxide value for the surface portions of 24 defective samples was 0.62 as compared to 0.26 for the interior portions. These values, however, were not as high as those obtained on butters which maintained their original quality during storage. On the limited number of samples examined, no definite correlation was apparent between the peroxide values of the

surface and the flavour defects which developed, or between the peroxide values and the pH and copper content of the samples.

Nevertheless, the data obtained did indicate that the surface butter had undergone more extensive oxidative changes than the interior portions of the butter.

DISCUSSION

From these studies it was difficult to determine whether the most important factor responsible for the development of undesirable flavours at the surface of the butter during storage was bacteriological or chemical. While the bacteriological data did not indicate any close correlation between total plate counts or counts of proteolytic, lipolytic or oxidase-positive organisms and the particular defect, they did show that many of the butters contained greater numbers of bacteria than should be expected with proper pasteurization and plant sanitation. The excessive numbers of bacteria found on the surface of some butters as compared to the interior brings up the question as to when this increase took place and whether it was due to recontamination or to the growth of bacteria already present in the butter.

During the life of storage butter there are two periods when conditions may be suitable for the growth of bacteria at the surface; one is before the butter is cooled to temperatures sufficiently low to prevent bacterial growth, and the other is during defrosting. In creameries not equipped with mechanical refrigeration, butter may be held for several days at ice storage temperatures which would permit the growth of bacteria, especially at the surface. Under such conditions, the bacteria might develop sufficiently to initiate the development of off-flavours. During defrosting, our own observations as well as those of McDowall (40) show that when butter is tempered at 68° to 70° F. the surface butter reaches a temperature suitable for bacterial growth in a very few hours. When organisms capable of causing flavour defects are present, they may increase to such an extent during the defrosting period as to be an important factor in the development of unclean flavours at the surface although the interior of the butter may not be affected. After packing, extraneous moisture is frequently present on the butter surfaces due to the method of finishing and from the wet liner. During defrosting, moisture may accumulate at the surface from condensation. This extraneous moisture not only reduces the salt concentration at the butter surface, thereby lessening its inhibiting power on bacterial growth, but provides conditions that are highly favourable for the growth of bacteria at the surface which are not found in the interior of the butter. As bacteria grow only in the aqueous phase, surface moisture is undoubtedly an important factor in surface deterioration of a bacterial nature. Knowing the importance of certain types of bacteria in butter deterioration, the data again point to the necessity of preventing recontamination of the surface butter during packing and finishing, and of getting the butter into cold storage as soon as possible to prevent the growth of any bacteria which may be present.

If the butters examined can be considered as representative of the bulk of Canadian butters going into storage, the data on pH values indicate the need for attention to neutralization to obtain a uniformly low acidity

in the cream and thereby a low acidity in butter intended for long holding. Too great a proportion of the butter had pH values lower than 6.7 to 6.9 which is considered optimum for storage butters. Proper acidity in the butter can only be obtained by close attention to all details in the neutralizing procedure, including strict accuracy in determining the initial acidity of the cream. While the copper content of the butters, with the exception of two samples, was less than the amount considered the maximum by some authorities for good keeping quality of butter during storage, they were higher than the minimum amounts necessary to induce oxidative changes in fat under favourable conditions. Even very small amounts of copper in combination with high acidity and undue exposure to light in salted butter are able to induce oxidation resulting in the development of undesirable flavours of a tallowy and fishy nature.

The whole problem of surface deterioration is a complex one. The objectionable flavours which develop at the surface of storage butter may be the result of bacterial activity or chemical changes, or a combination of both. Undoubtedly many of the surface defects of butter could be eliminated by guarding against recontamination and growth of undesirable types of bacteria at the surface of the butter, and by paying close attention to the factors which induce oxidation in the fat, the more important of which in salted butter are high acidity, contamination with copper and iron, and undue exposure of the cream and butter to direct or diffused sunlight.

SUMMARY

Bacteriological and chemical studies were made of a number of storage butters having surface flavour defects of a tallowy, stale, unclean and fishy nature to determine, if possible, some of the main factors responsible for such defects. A few samples of butter which had maintained their original grades were included for comparative purposes.

With the exception of 2 samples, all butters had storage periods of from 1 to 16 months at temperatures ranging from 15° to −20° F. The samples were representative of the butters made in the principal butter-producing provinces and the majority were manufactured during the summer months.

Three of the samples had total plate counts well over 1,000,000 per ml. and for about half the samples the total counts of the surface butter greatly exceeded the counts on the interior butter. A number of the samples, however, had total counts well below 25,000 per cc. on both surface and interior butter. The counts of proteolytic, lipolytic, and oxidase-positive organisms were relatively high in butters having high total counts and in a number of cases exceeded 100,000 per ml. at the surface. Relatively few or none of these types of organisms were present on plates from other samples.

Growth in litmus milk indicated that the general types of organisms were essentially the same on the surface and in the interior portions of the samples. The predominant morphologic types in most samples were gram-positive cocci but gram-positive and gram-negative rods were also regularly present.

There did not appear to be any correlation between either total bacterial counts or the numbers of proteolytic, lipolytic and oxidase-positive organisms and specific flavour defects. However, in many samples the numbers of organisms, especially at the surface, were greatly in excess of what might be expected in well-pasteurized cream butter manufactured under proper sanitary conditions.

For butters showing surface defects the pH values ranged from 5.56 to 7.56 with 31.5% below a pH of 6.05. All samples having fishy flavours had low pH values. With the samples which maintained original grade, all but one had pH values of 6.41 or higher, and 4 of the 7 samples had pH values of over 7.0.

The copper contents of the butters ranged from 0.07 to 0.8 p.p.m. but only 2 samples contained more than 0.2 p.p.m. of copper. With the exception of two samples, there was little difference between the copper content of the butters which lost grade for surface defects and those which maintained quality.

Peroxide values of the surface and interior portions of the butters were not excessively high, but indicated that oxidative changes were greater at the surface of the butter.

If surface defects of storage butter are to be kept at a minimum, care should be taken to prevent contamination and growth of undesirable organisms at the surface of the butter, to have the butter at the optimum pH values of 6.7 to 6.9, and to keep contamination with copper and iron at a minimum.

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TICK CONTROL WITH SPECIAL REFERENCE TO *DERMACENTOR ANDERSONI* STILES[1]

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Almost every year there are reported, from scattered points throughout south and central British Columbic, cases of tick paralysis affecting both man and animals. In the spring of 1941 a serious outbreak of tick paralysis in cattle occurred in the Kamloops district. This outbreak once again focussed attention on the fact that, so far, no adequate control measures for ticks or repellents as protection from their attack have been developed.

Ticks are cosmopolitan in their distribution, and tick control is a problem that has been studied in almost every part of the world where they are an economic factor in agriculture or public health. Practically all the control recommendations in the literature are deficient in their lasting effect. Range cattle, or sheep, need a protection lasting for at least two weeks as it is obviously not practical to round them up every few days for treatment.

Ticks have a complicated life-cycle. In the case of *Dermacentor andersoni* Stiles, the so-called wood tick or paralysis tick, three different hosts are required. The immature stages feed on small mammals or ground inhabiting birds. Tick control in nature would involve the destruction of all tick hosts, which would be a herculean task. In certain restricted areas tick populations can be, and are, kept down by rodent control, and these measures are also valuable agricultural practices. Generally speaking, however, it may be assumed that elimination of the natural tick hosts is out of the question.

Apart from tick parasites, which are effective only in limited geographical regions, ticks have few known natural enemies, which fact further complicates the problem of control. In India and Africa certain birds pick ticks off cattle, but they are in no sense an economic factor in control.

Tick control presents two problems. The first is that of protection of livestock from tick attack; and the second is treatment of livestock to kill, or cause removal, of ticks already attached. Sherrick (19) considers that repellency in a stock spray is more important than good knock-down and kill. This is certainly true where ticks are concerned, but much more information is needed about tick physiology and anatomy before much progress may be expected.

Extensive work on ticks over several years by various workers has resulted in a considerable accumulation of data on tick ecology, but there still remain several lacunae. In the meantime the demand for assistance

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in controlling ticks, and for a method of protecting livestock and humans from their attacks becomes more insistent, and emergency recommendations must be made.

Tick repellents

The development of an efficient repellent against arthropods is as much a problem for the biochemist as for the entomologist. Against certain insects marked success has been attained, although most of the publicized developments in this direction, such as mosquito lotions, afford only brief protection. Tick control recommendations have emanated from workers in Europe, Australia, South Africa and the United States of America, and are aimed at protection from specific tick pests of these countries. Climatic and geographical limitations, however, will probably rule out any universal tick control formula, as a plan that may be efficacious against ticks in one region may be unsuccessful when tried against different species in another part of the world.

Dip, Spray or Dressing

Assuming that a satisfactory and long-lasting repellent could be developed, its method of application must be considered, keeping in mind that the animals to be treated are range cattle and sheep. With certain species of tick, such as the Spinose Ear Tick, found in the southwestern United States, the pest usually locates in the animal's ears and in this case or in similar instances where the tick is localized in the site of its attachment, a dressing might prove to be the cheapest and easiest form in which to apply the repellent. Against ticks not localized in their point of attack on the host, a dressing applied to a limited region will not give complete protection. This was vividly demonstrated in a recent outbreak of tick paralysis near Kamloops, B.C. The cattle were all treated with a dressing composed of creosote, coal oil and raw linseed oil which was applied to the back of the head, neck and shoulders. Many of the treated cattle, however, became paralyzed, and ticks were found attached to the legs, belly and almost any part where there was no dressing. Obviously then, against *D. andersoni* Stiles, and ticks with similar habits, localized dressings are of little value.

Provided a lasting repellent could be found, dipping would appear to be the best method of application to ensure adequate protection for cattle. Dipping solutions could be applied in the form of sprays, but this is not practicable except in the case of very small herds unless a spraying machine or specially designed spraying chute is used. Spraying is less effective than dipping, is more time consuming, and is more expensive. Nevertheless, where only a few cattle are involved and where the loss of one or two steers would be a serious matter to the small operator, spraying may be recommended, although even with small owners it would be more satisfactory to dip.

Against *Dermacentor andersoni* Stiles, therefore, tick repellents or killing agents should preferably be applied in the form of a dip, but if no dipping vat is available, sprays would serve the purpose. Localized dressings are not considered satisfactory for protection from attack by ticks, but may have a place in killing ticks already present.

The protection of sheep from ticks presents special problems. Some operators believe that an autumn dip will have a lasting repellent effect that will still be effective in the spring. This has not been verified by controlled experiments. Spring dips, however, at least in British Columbia, are not advocated, as the lambing season almost coincides with the peak of the tick season. Avoidance of infested range in early spring, for the present at least, is the best insurance of freedom from tick injury.

Dusts

Many effective insecticides are applied in the form of dusts. Against some insect pests, dusts can be used when liquid insecticides are out of the question, such as in widespread outbreaks of forest insects or control of mosquito larvae in large swamps. Against animal parasites, however, dusts are less practical. A dog or other domestic pet, or a valuable show animal can be treated readily by dusting a powder into the pe age. Such a treatment is, of course, out of the question where a large number of cattle or a flock numbering hundreds of sheep is to be protected.

Medication to Prevent Tick Attacks

Various substances, administered either as a drench or with the feed, have been used to eliminate internal parasites from livestock. Against ticks, however, medication of the host animal so far has not proved to give any repellent effect. Nevertheless, every now and then one hears tales of livestock that graze on tick infested range, but which are never attacked because of substances administered to the animals with the feed. One of the substances widely credited in British Columbia with tick repellent qualities is sulphur. A letter received from a correspondent in March, 1941, says in part, "Every spring we put out a sulphur block of salt and find it keeps the ticks off the cattle; and the only spring we did not use it I had three cows die." Horses, too, are believed to be protected from attack by ticks if sulphur is administered with the feed. Because of this belief dealers in livestock feeds report a considerable sale of sulphurized salt blocks. Experiments at Kamloops in feeding sulphur to lambs have shown it to have no value in protecting them from attack by ticks. Bottle-fed lambs were given increasingly large doses of sulphur in their milk and when, after several weeks of this medication, ticks were placed on the animals, not only did the ticks attach readily, but one lamb was completely paralyzed as a result. In South Africa it has long been a common belief amongst farmers that cattle and other domestic animals, also poultry, can be kept free from ticks by giving them a daily ration of aloes. Bedford and Wilken-Jorden (3) have very thoroughly disproved this theory.

While medication cannot be ruled out as a means of protecting livestock against attack by ticks, and this method of approaching the problem cannot be discarded as hopeless, it must be admitted that so far, carefully controlled tests have not given very satisfactory results.

Livestock Sprays

The primary consideration in any repellent is that it will repel. It also should remain effective for a sufficient period of time to justify its use.

Most of the livestock sprays in use have an oil base in which is incorporated some toxic ingredient. According to Sherrick (19) the nature of the base oil affects the efficiency of certain toxic ingredients, and he found in some cases the same was true of the repellent effect. Melvin (14) found that petroleum oil cattle sprays caused physiological disturbances in cattle, especially a heavy, almost non-volatile oil. Freeborn *et al.* (7) also noting physiological disturbances from petroleum oil sprays observed that oil-water emulsions plus pyrethrum extracts were as effective in fly repellency as the undiluted oil spray and caused little physiological disturbance.

Many so-called "cattle sprays" or "stock sprays", according to Sherrick (19) are merely cheaply made household insecticides, quite unsuitable for use on the body of an animal.

Arsenical Dips

Arsenical dips are most commonly recommended for the killing of ticks on livestock. In the early days of the fight against the Texas fever tick, Ransom and Graybill (16) investigated many arsenical dips and a few years later Ellenberger and Chapin (6) report that "Proprietary arsenical cattle dips appear now to have passed the experimental stage." In other parts of the world also arsenic is regarded as the most effective agent for the destruction of ticks and arsenical dips are widely advocated. Sanderson (18) in Australia, Bedford (2) in South Africa and Macleod (13) in Britain, all comment favourably on arsenical dips. The protective effect of arsenical dips against reinfestation by ticks remains in doubt. Ransom and Graybill (16) note that "little evidence has been obtained as to the degree of protection against reinfestation afforded by arsenical dips or as to the length of time after treatment that such protective action continues. It seems, however, that cattle are protected from reinfestation, at least to some extent, during several days after treatment." The Council for Scientific and Industrial Research, Commonwealth of Australia (1) tried to determine the extent, if any, of the protective action of standard arsenical dips against reinfestation. Treatment with the prescribed standard arsenical fluid afforded protection against reinfestation only for a period of two days. Jack (11) claims that arsenicals act as contact poisons to ticks, which if true, may account for some protective effect. For ridding livestock of ticks already present, arsenical dips still appear to be the best recommendation. In Russia the tick *Dermacentor marginatus* Sulz. is the vector of a serious disease of horses. Shmulevich *et al.* (20) state that the tick was controlled and disease incidence lowered 90% by rubbing down the horses every five days in May and June with a solution of sodium arsenite in water. This recommendation is impractical for range conditions in this country.

Organic Compounds

Among the recently developed insecticides for the control of phytophagus insects are certain organic compounds such as dinitro-cyclo-hexyl-phenol, 3,5 dinitro-o-cresol and related compounds. Such insecticides have proved to be effective against insects and apparently do little or no damage to the plants in the dilutions used. They are equally effective against

ticks, but unfortunately are highly toxic to the tick host. If it can be ascertained that high dilutions of such materials have a practical acaricidal value and still are too dilute to act as stomach poisons, such materials may have a place in tick control. However, a great deal of work is necessary on the physiological action of such compounds before they can be recommended. Certain thiocyanates are marketed under trade names as ingredients for livestock sprays, and have proved effective against flies. They have not yet been used in the field against ticks although in the laboratory they show promise.

Certain plant extracts such as derris and pyrethrum would appear to be ideal as acaricides. They are highly lethal to insects and ticks also, and are relatively non-toxic to mammals. The chief factors that might preclude their use in large scale dipping operations would be cost, which is high, and lasting quality, which is not great. Derris is mentioned favourably by Gunn (9), who reports much success in protecting cattle from ticks by applying warble fly wash over the shoulders and around the back of the head of cattle. The Council for Scientific and Industrial Research (1) in Australia sprayed moderately infested animals with derris extract and potash soap and compared the results with a similar number of untreated controls. "The result established the fact that although the derrisine solution kills a considerable proportion of the ticks, it has no practical value as a tick destroying agent." Mironov *et al.* (15) found that nymphs of two species of *Ornithodorus* were not affected by derris powder, but larvae and nymphs of *Ixodes ricinus* L. were killed by it in from one-half to two hours. These workers also had good results with water extracts of derris powder in soapy solution against larvae of *I. ricinus* L. Macleod (13) reports that "derris in solution does not long retain its parasiticidal properties." Bishop and Smith (4) advocate a thorough application of derris as a powder or wash for destroying ticks already attached to dogs and to prevent reinfestation for short periods with the wash having a more extended repellent action. Strong (21) reports that workers in the United States Bureau of Entomology and Plant Quarantine had success with derris against ticks. Derris powder containing 1 to 3% rotenone killed all stages but the engorged females of *Dermacentor variabilis* Say, and four to nine ounces of derris were sufficient to control the winter tick *D. albipictus* Packard on an adult horse or steer. Harwood (10) recommends a bath of derris powder in soapy water for controlling ticks on dogs. Ross (17) in Australia found that a weekly application, either of derris powder in dry form or as a 1% soapy cold water infusion, conferred a high measure of protection against tick infestation. Mail and Gregson (unpublished) found that ticks will readily infest lambs treated heavily with derris powder and that although many ticks were killed, a few succeeded in attaching and in some cases in engorging. It would appear, therefore, that while a derris dip is of value in killing ticks already on the host, as a protective repellent for range stock its lasting quality is doubtful. For the treatment of domestic pets derris should be most satisfactory.

Workers in the United States Department of Agriculture are reported by Strong (22) to have had some success in controlling dog ticks, *Dermacentor variabilis* Say, by spraying the vegetation harbouring them with nicotine

sulphate. This recommendation would be only likely to have possible value in British Columbia in densely settled resort areas where domestic pets are one of the chief sources of maintaining a large tick population.

Use of Sheep as Tick Traps

It is claimed by some ranchers that the natural oils in sheep wool are effective in killing ticks. The use of sheep, however, as a tick control measure is not practical as was demonstrated by King (12) in Montana in 1915. His experiment led him to the conclusion that in spite of numerous dead ticks found in the fleece, enough ticks could mature on sheep to maintain a normal population. In this connection, breeds of sheep are said to differ in their susceptibility to injury from ticks on account of the variation in lanolin content of the fleeces. Long-haired varieties, with a heavy coating of grease near the skin, apparently have in this lanolin a certain degree of protection as at shearing time dead ticks often are found in this region. However, as experiments have shown that lanolin possesses neither repellent nor toxic effects for ticks, it must be concluded that death is due to physical entanglement or suffocation in the dense greasy fleece.

Limited Time during Which Experiments Can Be Performed on Feeding Ticks

It has been noted in studies in British Columbia, that when ticks (*Dermacentor andersoni* Stiles), first come out of hibernation in spring, they will attach readily and feed on their animal hosts. After about three months, however, they show less ability to feed and by autumn, although they may still attach, they seldom are able to engorge. This physiological trait, for which no reason has yet been found, limits the period during which experiments on feeding ticks can be performed. It may, therefore, be necessary to learn what stimulus is required to induce ticks to feed on laboratory animals throughout the year, whether temperature, humidity, ultra-violet light or some combination of these three or other factors. Pending the development of some treatment or technique to overcome this inability to feed on the part of the tick, experiments are limited to the brief period during the early part of the season when ticks are normally active.

Parasites

No discussion of tick control measures would be complete without some reference to the possibility of parasitic control. Tick parasites, usually Chalcids, are sometimes found in nature and as they are specific they might be considered of value in control.

At least three major experiments in the use of tick parasites have been conducted through several years in North America, one at Naushon Island off the Massachusetts coast, another on Capers Island, South Carolina, and a large-scale operation in Montana. For various reasons these experiments have been inconclusive if not actually without success. In Montana, the inability of the parasites to establish themselves may have been due, in part, to climatic conditions. The continental type of climate prevailing over most of Montana, with great extremes of temperature in winter and summer, differs considerably from that prevailing in regions where the parasite is native, and this may have been an important factor in the failure

of the parasite to establish itself. The influence of the Montana climate on the parasites is discussed briefly by Cooley (5), who has contributed so much to our knowledge of tick-parasite relations. According to Bishop and Smith (4) excessive dryness is destructive to all stages of the parasite, so that differences of precipitation may well affect the ability of the parasite to become established. It is doubtful, therefore, with *Dermacentor andersoni* Stiles at least, whether the dry interior of British Columbia and other regions where this tick abounds would permit of any greater success in establishing this parasite than was obtained in Montana. Control by parasites of the tick *Ixodes californicus* Banks, common to the Pacific coast, where climatic conditions are so different to those prevailing over the arid interior and presumably more favourable to the parasite, may be a distinct possibility.

Tick Immunity

There is a strong belief among many ranchers that sheep constantly exposed to attack by ticks in the infested regions of British Columbia, develop some degree of immunity. A rancher in the Kamloops, B.C. area brought in 1300 sheep from a section of Alberta where ticks were absent. Next spring 100 sheep were paralyzed by ticks. Bands of locally raised sheep in the same locality were unaffected or suffered very minor casualties. A similar case is on record in the files of the Kamloops Laboratory involving beef cattle. A rancher at Baynes Lake, B.C., in 1935 lost 16 head of cattle from tick paralysis. He reports "I may say that this bunch I lost were all out of a car load I shipped in here the last of February. The others I have had over a year were not bothered so much, nor did any die." The validity of such implied claims of acquired immunity to tick attack is open to doubt as so many factors are involved. However, there is certain experimental evidence in support of the immunity theory. Ross (12) claims that a considerable number of dogs in areas infested by the tick *Ixodes holocyclus* Neumann are in possession of a very high degree of immunity, and his experiments suggest that this immunityis wholly acquired and is not inherited. Ross further suggests that the serum of immune animals appears to be useful as a curative agent for the treatment of dogs affected by tick paralysis. Trager (23) showed that "an infestation of guinea pigs or rabbits with larvae of *Dermacentor variabilis* induces an acquired immunity which effectively prevents subsequent batches of larvae from engorging." In a later paper Trager (24) discusses the degree of immunization conferred on guinea pigs by injections of extracts of specific glands or portions of ticks. Gregson (8) reports preliminary work in 1936 to test the immunity theory, using *Dermacentor andersoni* Stiles and guinea pigs. In the same paper Gregson also notes a very decided immunity exhibited by fitches against *Ixodes texanus* Banks. From all these observations it may not be too much to hope that out of this study of immunity there may be developed protective serums against the harmful effects of tick bites, that will revolutionize our present attempts at tick control.

SUMMARY

Tick paralysis of man and animals is common in British Columbia. No adequate tick control is known. The most important need is for a good repellent that will be effective for at least two weeks. Localized

dressings are of little value against *Dermacentor andersoni* Stiles. For cattle it is considered that a repellent substance is best applied as a dip, or a spray if a dipping vat is not available. For sheep, avoidance of infested pastures in spring at present is the only good protection against injury by ticks.

It is not considered that repellents applied in the form of dusts are practical for range herds.

Medication of the host by ingestion of such materials as sulphur to protect against attack by ticks, so far, has not given satisfactory results.

Many of the cattle sprays on the market have an oil base and in many cases such sprays are quite unsuitable for the purpose. Oil emulsions probably would be less harmful and as effective.

Arsenical dips are still the best recommendation for ridding stock of ticks. Some of the 2,5 Dinitro-6-R-Phenols have potential promise as acaricides but more knowledge of their physiological action on mammals is needed before they can be recommended. Thiocyanates may prove effective on the basis of laboratory trials.

Reports on the efficacy of derris against ticks are conflicting. It seems generally agreed that derris is effective for a short time, but that frequent treatments are necessary. In the Eastern United States spraying of the vegetation with nicotine sulphate has been used against *Dermacentor variabilis* Say.

Sheep as tick traps are of no value and the lanolin of sheep wool is not toxic to ticks.

In British Columbia experiments on adult ticks are limited to the few spring months when ticks are actively feeding.

Tick parasites may have a place in the control of the Coast tick, *Ixodes californicus* Banks, but are not considered promising against *Dermacentor andersoni* Stiles of the dry British Columbia interior.

Host animals can acquire a definite immunity to attack by ticks.

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